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MICROAGGREGATES: EXPERIMENTAL AND CLINICAL ASPECTS - SYMPOSIUM --ETC(U)
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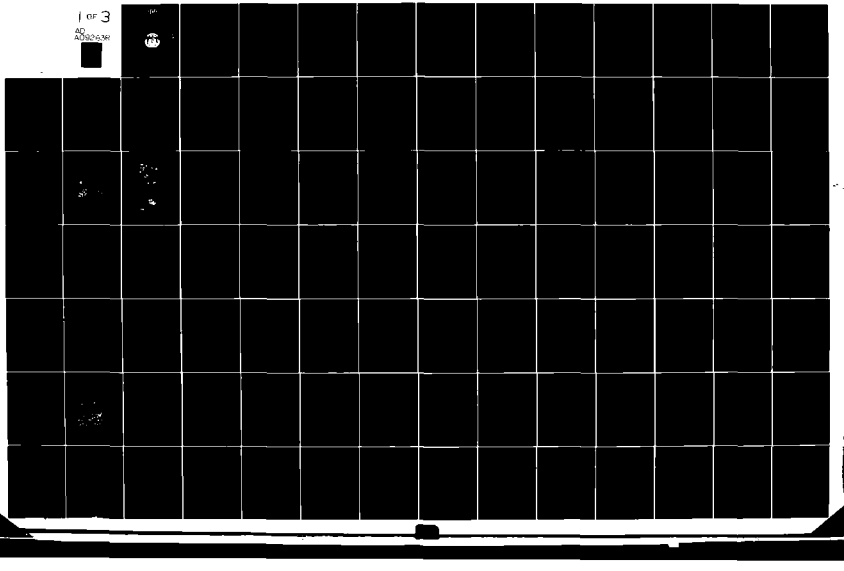

LEVEL II

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MICROAGGREGATES

EXPERIMENTAL AND CLINICAL ASPECTS

UNITED STATES ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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MICROAGGREGATES: EXPERIMENTAL AND CLINICAL ASPECTS

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Symposium on Microaggregates held at Letterman Army Institute of Research on 20-21 June 1977

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PREFACE

Under the auspices of the United States Army Medical Research and Development Command, a symposium on microaggregates was held at the Letterman Army Institute of Research on June 20-21, 1977.

The purpose of the meeting was to investigate the nature and significance of microaggregates and microemboli in the pathogenesis of clinical disease states.

During the Vietnam conflict, the association of posttraumatic pulmonary insufficiency with multiple transfusions gave impetus to the investigation of microaggregates as a potential etiologic factor. ~~Studies presented during~~ the first session ~~of the symposium~~ (June 20, 1977) deals primarily with this question. Various laboratory methods are presented which attempt to create a valid animal model for microaggregate-induced pulmonary insufficiency. other studies investigate the potentially deleterious properties of banked blood, including in vitro formation of microaggregates, alterations in rheologic properties, and the inclusion of suspected noxious materials (phthalates) in transfused fluids.

The second session (~~June~~ 21, 1977) ~~is~~ devoted to more clinical aspects of microaggregates. The structure and composition of the aggregates is demonstrated, and methods of detection, quantification, and removal reported. In vivo formation of aggregates and microthrombi as a result of platelet hyperaggregation is discussed, and pharmacologic means for inhibiting this phenomenon delineated. The role of microaggregates in producing clinically apparent disease in man is further discussed.

To each of the contributors is due sincere thanks. The continued support of MG William S. Augerson, Commander, United States Army Medical Research and Development Command, and COL LeeRoy Jones, Deputy Commander, United States Army Medical Research and Development Command made the completion of this symposium a reality.

Special thanks is given to Mrs. Judy A. Pereschuk and Ms. Terri Baker for their efforts in preparing the manuscripts. A special recognition is given to Ms. Linda Richardson for her participation in the preparation, organization and dedication to the completion of the entire editorial process.

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SESSION I

June 20, 1977

Letterman Army Institute of Research

San Francisco, California

PULMONARY INSUFFICIENCY FOLLOWING MASSIVE TRANSFUSION: THE EXPERIENCE IN VIETNAM

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The combat experience in Vietnam led to large numbers of heavily transfused casualties, some of whom were studied by the various research teams during and after resuscitation. Fine filters were not then available to test the hypothesis that microaggregates in the stored blood were contributing to postresuscitative pulmonary insufficiency, but considerable data were accumulated on the recipients of large amounts of grossly filtered blood beyond the second week of storage. If microaggregates cause significant pulmonary insufficiency, an effect should have been seen in these casualties. Several teams have published their experience, largely as the occurrence and degree of hypoxemia following transfusion. Unfortunately, the results seem to be contradictory. We will review some of these reports and present some new, pertinent data on combat casualties. The question, however, will remain largely unresolved.

The first report indicating that transfusion was associated with hypoxemia in combat casualties in Vietnam was by Simmons et al. in 1969 (1). In a study of 225 combat casualties they found a statistically significantly lower arterial oxygen tension (PaO_2) on the 2nd and 4th days after wounding in those who had received over 8 units of blood. They also found that the location of the wound and the presence of shock on admission were associated with lower postresuscitation PaO_2. The data regarding transfusion are not given in tabular form and are not divided by type of injury. In 1970, Moseley and Doty (2) reported extensive embolic debris in the lungs of two massively transfused casualties, and they postulated a causal connection between transfusion and pulmonary insufficiency. Later that year McNamara, Molot, and Stremple (3) presented the best defined statistical relationship between transfusion and hypoxemia. They found in 218 casualties that those with major injuries had lower PaO_2 in the 48 hr after wounding than those with minor injuries, and that the degree of hypoxemia was proportional to the amount of blood transfused. Twenty-seven casualties receiving less than 5 units of blood had mean PaO_2 in the first 48 hr after injury not statistically significantly different from those with minor injuries, whereas 26 who had received more than 5 units of blood were significantly more hypoxemic, and this was a bit worse in 13 who had received over 10 units of blood. Among casualties in shock on admission, 6 who received more than 10 units of blood were significantly more hypoxemic than the 5 who received less. Again, the patients were not subdivided according to type of injury.

On the negative side, Collins et al. (4) in 1968 reported 69 casualties with only peripheral injuries studied before the administration of general anesthesia. In this group, the opposite effect was seen; those who received more blood before operation had higher PaO_2, presumably from hyperventilating in response to a metabolic acidosis. This study was controlled for the location of the wound and the effects of anesthesia, but did not extend into the convalescent period. Martin et al. (5) in 1969 reviewed 100 autopsies on combat casualties and found little evidence of embolic material in the lungs related to massive transfusion, but the study was a retrospective one, and it is not clear how long the intervals were between transfusion and death.

All of the studies mentioned so far were by Army research teams. The Naval surgical research teams working at the same time have not reported a relationship between transfusion and postresuscitative hypoxemia. Carey et al. (6) specifically noted a lack of such relationship, but did not give data. Proctor et al. (7) studied Marine casualties in ventilatory failure, and did not mention massive transfusion as a contributing cause. Like most of the teams, they found massive direct injury to the lungs, major infection, renal failure, and overloading with fluids to be the commonest causes of severe pulmonary insufficiency.

Bothered by some of these inconsistencies, we reviewed our data (8) in 1969 to assess the relationship between hypotension and hypoxemia on admission, and again found a relationship to the location of the wounds. We are now examining the accumulated data of the first three Army surgical research teams in Vietnam for the relationship between transfusion and hypoxemia. This includes the casualties already reported by Collins et al. (4) and by Simmons et al. (1) plus an additional several hundred casualties. The analysis includes the amount of blood transfused and the type of wound.

The data we are reporting today deal only with PaO_2 on these casualties before the administration of general anesthesia. With the interval of 10 years since the original data were gathered, there is some unfortunate uncertainty of detail. The temporal relationship of the initial blood sample to the transfusion is not clear for approximately 40 casualties, and to anesthesia for another 10. The data are presented in two forms. Assumption A is that all received the blood before sampling. This is very unlikely. Assumption B is the more likely, that none received the doubtful transfusions before sampling. Completely eliminating the 50 uncertain data yields results very like Assumption B. For the majority, the relationships are clear. Only one real difference results as a consequence of the two interpretations.

All casualties are considered only according to whether transfused or not (Table 1). There is no statistically significant difference between them. They are divided in Table 2 according to type of wound, with injuries classified as penetrating the chest and/or abdomen ("Trunk") versus all others ("Nontrunk"). Burns and major injuries to the brain are excluded. In both assumptions, there is a highly significant difference (Student t-test for unpaired data) between the mean PaO_2 in categories of wounds in both the transfused and nontransfused groups ($P = .001$). The differences between transfused and nontransfused casualties within categories of wounds are not significant in either assumption. In table 3, the data are further divided according to whether transfused more than 5 units, or less. The numbers in sub-groups are now small, but for the first time a statistically significant difference appears that is related to transfusion. Under Assumption A, those with nontruncal injury transfused over 5 units of blood had significantly lower PaO_2 than those not transfused ($p = .05$) or those transfused 1-5 units ($p = .01$). Under Assumption B, there are no significant differences among transfusion groups. There are no differences that are related to transfusion among those with truncal injury in either assumption.

Table 1. Mean arterial oxygen tension (mm. Hg ± 1 SD), and number of casualties (n) according to whether transfused before sampling; admission values before anesthesia. See text for explanation of Assumption A and Assumption B.

A		B	
Not Transfused	Transfused	Not Transfused	Transfused
84.03 ± 17.12 n = 314	82.99 ± 17.82 n = 102	84.19 ± 17.41 n = 373	81.54 ± 15.88 n = 57

Table 2. Mean arterial oxygen tension (mm Hg ± 1 SD), and number of casualties (n) according to whether transfused before sampling and to type of injury; admission values before anesthesia. (See text for definition of truncal and nontruncal injuries, for explanation of A and B, and for statistical analysis.)

A

	Nontruncal injury	Truncal injury
Not transfused	89.33 ± 14.04 n = 194	76.58 ± 17.55 n = 122
Transfused	90.43 ± 16.34 n = 48	76.39 ± 16.55 n = 54

B

	Nontruncal injury	Truncal injury
Not transfused	89.93 ± 14.74 n = 213	76.94 ± 17.47 n = 155
Transfused	88 ± 12.98 n = 30	74.37 ± 15.94 n = 27

Table 3. Mean arterial oxygen tension (mm Hg ± 1 SD), and number of casualties (n) according to whether transfused before sampling and to type of injury; admission values before anesthesia. (See text for definition of truncal and nontruncal injuries, for explanation of A and B, and for statistical analysis.) Subdivided according to quantity of transfusion.

A

	Nontruncal injury	Truncal injury
Not transfused	89.33 ± 14.04 n = 194	76.58 ± 17.55 n = 122
Transfused 1-5 Units	93.32 ± 14.18 n = 39	74.72 ± 14.95 n = 41
Transfused > 5 Units	77.89 ± 19.89 n = 9	81.62 ± 20.64 n = 13

B

	Nontruncal injury	Truncal injury
Not transfused	89.93 ± 14.74 n = 213	76.94 ± 17.47 n = 155
Transfused 1-5 Units	88.91 ± 9.88 n = 22	72.91 ± 16.19 n = 23
Transfused > 5 Units	85.5 ± 19.87 n = 8	82.75 ± 13.1 n = 4

These findings do not support a major role for transfusion in the genesis of posttraumatic pulmonary insufficiency. The one statistically significant change occurred only with the less likely of the interpretations of our unclear data points. This relationship was also in the "wrong" group. The patients with truncal injury had significant hypoxemia from the injury alone. One would expect any additive insult, especially microembolization, to be more evident in such patients than in those with more normal pulmonary function, but such was not the case.

There are a number of obvious limitations in this analysis. First, this is essentially a retrospective study because none of the three teams was studying the effect of transfusion on pulmonary function at the time the study was carried out. At various times there was special interest in blast amputations, femoral fractures, penetrating thoracic injuries, thoracodorsal

injuries, and others. At all times, however, there was special interest in massive blood loss and hence indirect attention to massive transfusion. The selection of patients then was somewhat uneven, but included most of the extensively transfused. The second potential problem is the end point, arterial oxygen tension. For microembolization, this seems to be appropriate, as almost every substance used to produce microembolization to the pulmonary vascular bed has been found to produce concurrent hypoxemia which varies in degree with the amount of material injected (9). The third problem is the limited and early time of sampling after transfusion. Microembolization to the lungs produces hypoxemia very rapidly, but with some forms, such as neutral fat, a later form of hypoxemia occurs concurrent with delayed hemorrhagic consolidation (9). There has been no evidence of such a lesion from transfused blood, but it is not inconceivable. The early period of sampling was chosen to eliminate the variables of anesthesia, operation, and late complications. We are now attempting an analysis of these data including studies for several days after transfusion, where available. The final problem is the most serious. There are many potential causes of hypoxemia in the seriously injured, massively transfused casualty. We tried to avoid some of these by the early preanesthetic studies. Even so, the most extensively transfused casualties were often the most massively wounded. This variable would, if anything, favor the concurrence of hypoxemia and extensive transfusion, but such a relationship was only minimally evident in this study. To answer the specific questions concerning microembolization, a prospective, randomized study employing fine filters would have been preferable. The absence of significant hypoxemia related to transfusion without fine filters makes it unclear that a beneficial effect could have been found.

The casualties studied were vigorous young men, preselected for good health and athletically conditioned. On the other hand, many had serious direct mechanical injuries to the lungs, and despite preexisting hypoxemia in those circumstances, they showed little if any further impairment from transfusion. The blood that was used was almost all over 10-14-days old because of the excellent supply and careful management of inventory at serial points of distribution, so that microembolic material should have been even more extensive than that found on average in civilian practice.

We conclude that if transfusion contributes significantly to pulmonary dysfunction, it must be primarily a delayed lesion. This is not consistent with what is currently known about pulmonary microembolization with a variety of materials.

There is an additional perspective on the experience in Vietnam that should be considered. Mendelson (10) reviewed the various types of data that are available regarding the quantities of blood transfused in Vietnam. These data range from various

inventory control records, to questionnaires sent to specific locations, to direct observations by various investigators, and include facilities of both Army and Navy. None are ideal, but there is a reassuring consistency in the data obtained from widely differing sources and gathered at different times. The percentage of casualties who required transfusion ranged from about 25% by one method of reporting to about 45% by another. By any method, the total number of casualties transfused in Vietnam probably exceeded 50,000/year in the peak years of the war.

The mean number of units of blood transfused per casualty transfused ranged from 3.7 to 8.9, with most values between 4.3 and 7.2 units. These mean values deal with a skewed distribution, however. Hardaway gathered the only sizable number of reports on the number of units of blood actually given to individual transfused casualties. According to those data, 34.3% of 2774 transfused casualties received over 5 units of blood, and 13.8% received over 10 units. This pattern is consistent with our direct observations. It is, therefore, possible to estimate that over 15,000 casualties received over 5 units of blood, and over 5000 casualties received over 10 units of blood/year during the peak war years (1968-1970). There are no adequate data on the rate of pulmonary insufficiency in these combat casualties. Several observers have estimated that about 1-2% of all casualties might have required ventilatory assistance. Most of these had direct injury to the chest, overloading with fluids, or remote infection. The numbers that were unexplained were very small, yet the numbers "at risk" from extensive transfusion were quite large. This proves very little because such a direct relationship was rarely sought, and the deleterious effects of transfusion may well be more additive than direct, but it probably does help to indicate what extensive transfusion of blood not filtered for microaggregates does not do.

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IN VITRO FORMATION OF MICROAGGREGATES

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Clinicians have long recognized that a standard transfusion filter will frequently develop large amounts of amorphous soft clot and "debris" after infusion of several units of stored blood. Mosely and Doty (1) measured up to 4 g wet weight of such material per unit of stored blood. Swank (2) called attention to this phenomenon in 1961, reporting some studies on the composition of such debris and describing a semiquantitative method for measuring microaggregates in stored blood, the screen filtration pressure (SFP). Virtually all subsequent studies have confirmed the finding of "debris" or microaggregates in stored blood (3) and major research interests have developed with emphasis on several questions regarding this phenomenon:

1) What is the mechanism by which such microaggregate formation occur?

2) Can this microaggregate debris be removed from stored blood?

3) Does the transfusion of large volumes of blood and the resulting infusion of microaggregate debris have any pathological significance?

It is the first question with which the current report is concerned.

Identification and Quantification of Debris Accumulation in Stored Blood

Screen filtration pressure was the first method used for studing microaggregates in stored blood (3). It provides an indirect measure in that it estimates debris concentration from the pressure generated by passing blood at a constant flow rate through a 20μ mesh screen. Although an indirect index, it still provides a reasonably accurate expression of microaggregate content of stored blood and continues to be useful in clinical studies. Modification of the SFP method has been reported attempting to provide a more

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direct, quantitative [illegible] estimate by weighing the fine mesh screens before and after passing fixed quantities of blood through them (4). The [illegible] obtained was no different qualitatively than that obtained from SFP measurements and was not as reproducible.

Both light and electron microscopy have been useful in identifying microaggregate formation and following development of microaggregates during the course of blood storage (5, 6). In this regard, it has been particularly useful in studying the pathogenesis of debris formation and studying microaggregate morphology through the early phases of microaggregate formation.

Most recently, measurement of microaggregates has been performed with an electronic particle counter (7, 8). This method has proven most valuable for identification of microaggregates formed in vitro in banked blood, but as will be discussed elsewhere, is not as accurate for measuring microaggregates in vivo (8). The method has been particularly useful in following the formation of microaggregates in stored blood and studying microaggregate population shifts during the storage period.

Microaggregate Formation

Significant quantities of microaggregates are detectable by all methods within 24 hr of storage of whole blood in citrate phosphate dextrose (CPD) or acid citrate dextrose (ACD).

The actual volume of particles as illustrated by SFP and electronic particle counting (Figs. 1a and b) increases steadily during the storage period (8-10). In addition, there is a progressive decrease in the number of microaggregate particles consequent to a shift in the population toward progressively larger particle size (8).

Early studies on microaggregate formation indicated that they were composed principally of platelets and white cells, primarily leukocytes (2, 11). Subsequent studies have identified increasing incorporation of fibrinogen into the microaggregates beginning about the tenth day of storage and continuing thereafter (9) (Figs. 2a and b).

The precise mechanism of in vitro microaggregate formation is not known. It is evident that the platelet is central to the process and initiates microaggregation. We have previously demonstrated delay in microaggregate formation and marked reduction in microaggregate quantity by pretreating donors with aspirin, a drug known to interfere with platelet aggregation (12) (Fig. 3).

DEVELOPMENT OF DEBRIS DURING STORAGE

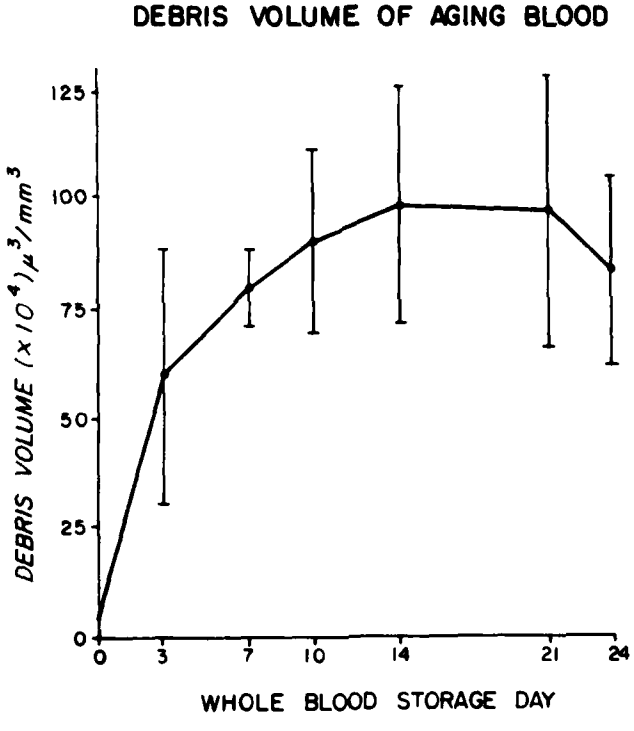

Figure 1a
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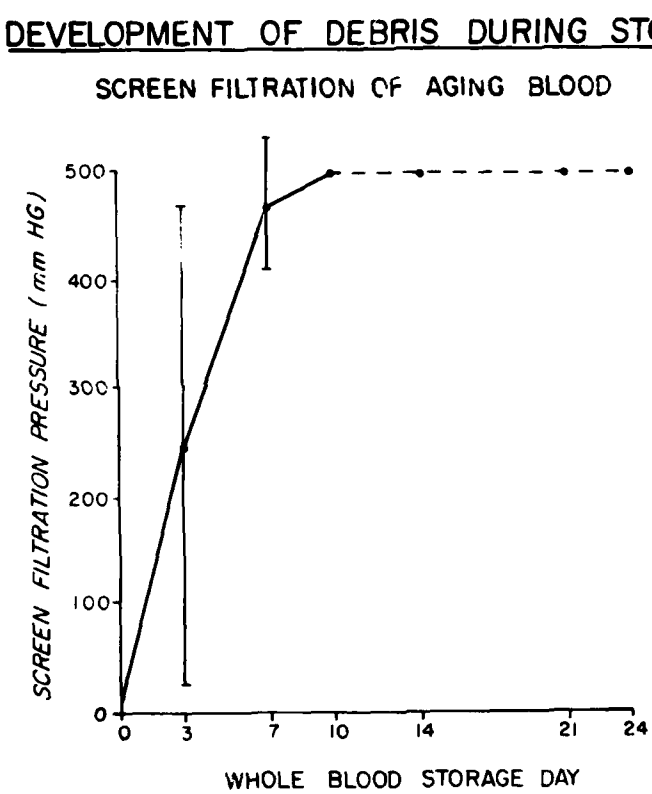

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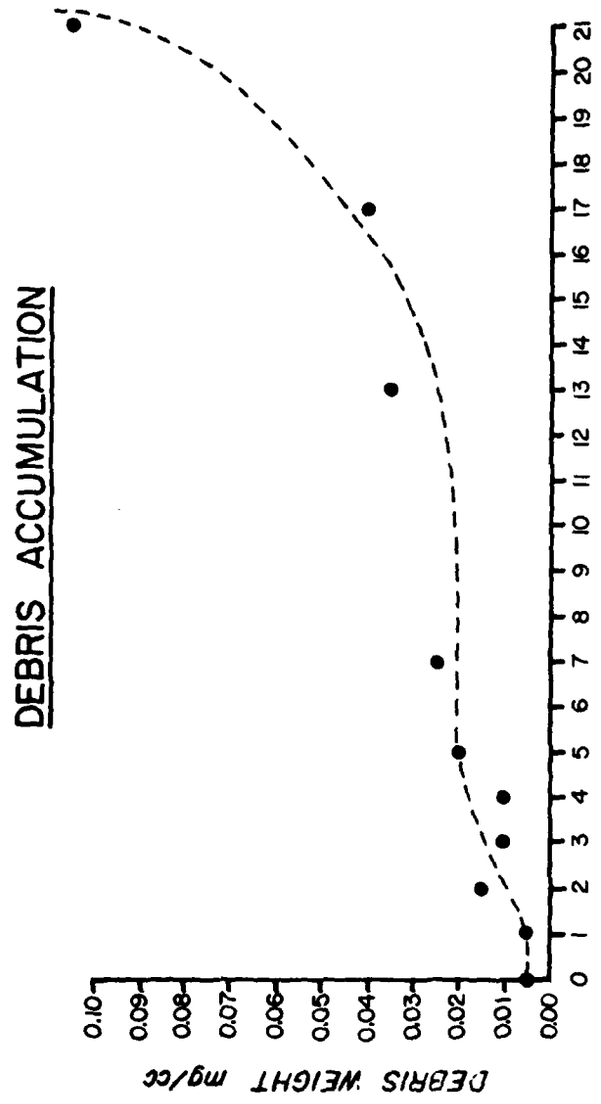

Figure 2a
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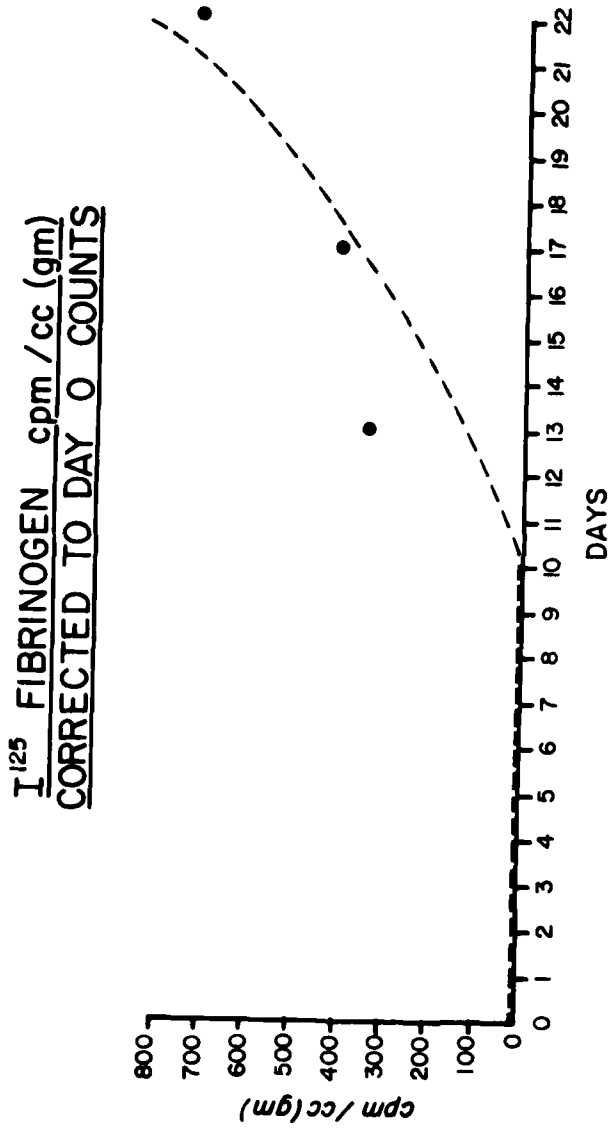

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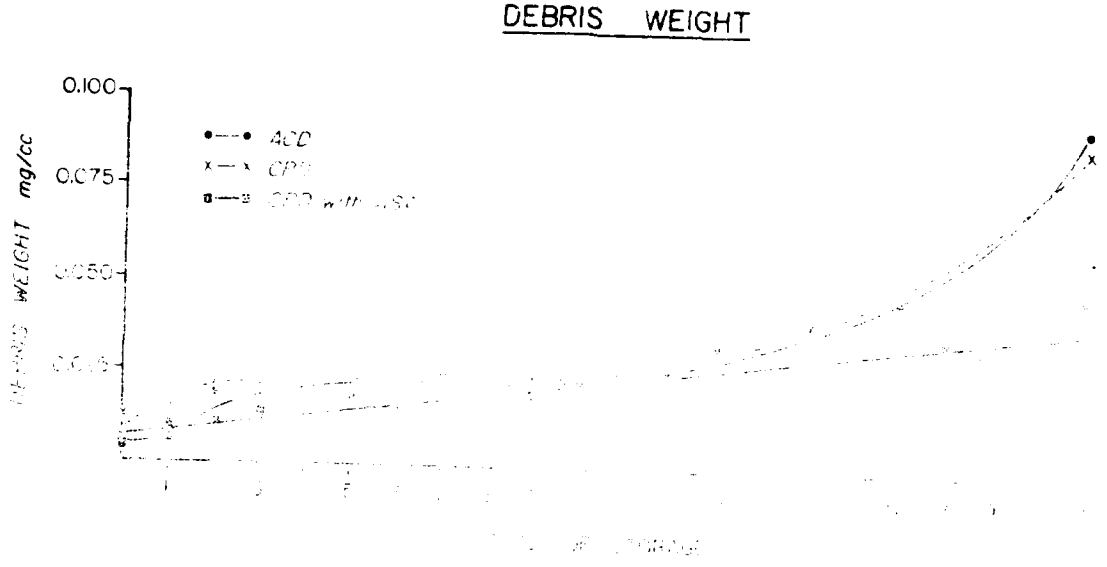

Figure
With permission by Surgery, Vol. 76, No. 2, 197.

Microscopically, well-established platelet aggregates with attached leukocytes are evident in stored blood as early as the first day of storage and increase progressively in size during the storage period (6) (Fig. 4). Changes in microaggregate morphology also occur with the microaggregates becoming more tightly packed with a dense amorphous central area. The morphologic changes occur concomitant with disruption of white cells and progressive deterioration in the ultrastructural morphology of platelets not involved in microaggregates (Fig. 5).

Microaggregates in stored blood are "stable" particles. There is a persistence of microaggregates following dilution for particle counting (8) (Table I). This is in contrast to platelet microaggregates formed in vivo or in response to ADP, collagen or epinephrine which deaggregate completely with sample dilution (8).

Table I

CORRELATION OF SFP TO DEBRIS VOLUME AND POPULATION

COMPARISON OF SFP TO:		SAMPLE POPULATION n	CORRELATION COEFFICIENT r
WHOLE BLOOD	12.7-80.6μ	16	.929
DEBRIS VOLUME	20.2-80.6μ	17	.646
WHOLE BLOOD	12.7-80.6μ	17	.565
DEBRIS POPULATION	20.2-80.6μ	17	.808

The specific underlying events in stored blood which precipitate microaggregate formation are unknown. Loss of cellular ATP (13) with accumulation of ADP in the serum, progressive losses in energy substrate (14), mechanical trauma to the blood (9), cooling the blood to 4°C (15) and a variety of other physical and chemical changes which occur in stored blood are probably all contributing factors (16).

Discussion

It is evident that whole blood, stored by currently used preservation methods, develops progressively larger quantities of microaggregates. The platelet appears to be a key element in the initiation and formation of microaggregate particles. The specific storage events which initiate platelet aggregates in blood are not

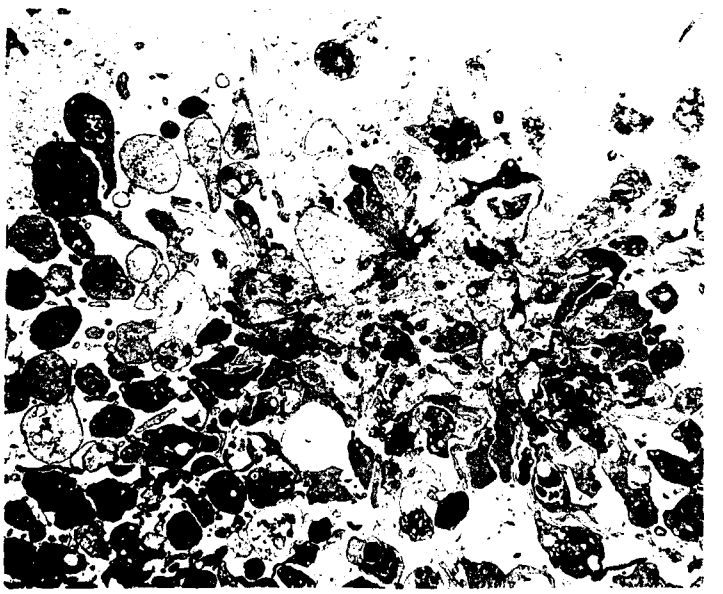

Figure 4. Microaggregates in stored blood - day 1 of storage

Figure 5a. Five days x 9,000.

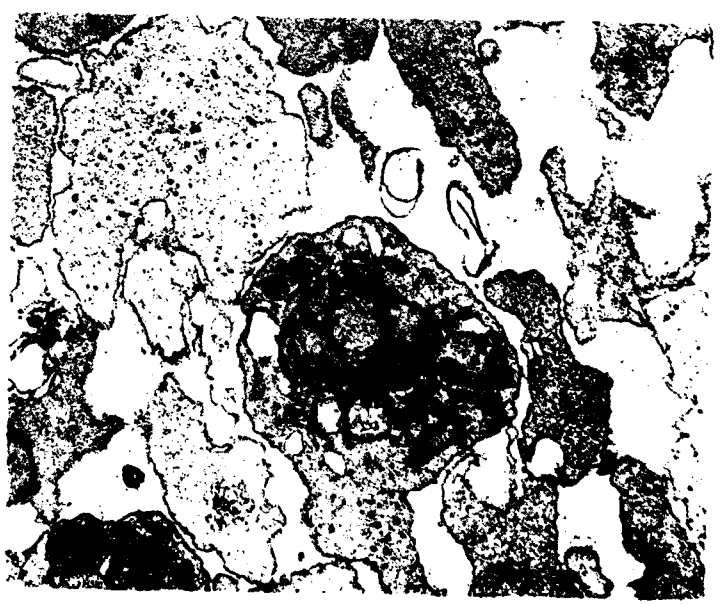

Figure 5b. Ten days x 27,000

known. White cells and fibrinogen also play a role in microaggregate formation and may contribute to the peculiarly stable nature of the microaggregates when subject to progressive dilution as compared to platelet aggregates, formed by the addition of an aggregating agent to platelet rich plasma.

This particular physical property serves to differentiate microaggregates formed in stored blood from those developing in vivo or in a pump oxygenator system. It further emphasizes that studies relating to microaggregate prevention or removal, or studies relating to the pathologic significance of microaggregates must be directed at the specific type of microaggregate under investigation.

In addition, we have demonstrated significant species differences in platelet aggregation and microaggregate formation in stored blood of dogs, monkeys, and man. This information suggests that studies on the formation and clinical significance of microaggregates are best performed in humans whenever possible.

When other species are used, studies of platelet function and microaggregate formation should be undertaken to assure that baseline values are similar to that seen in humans.

Because of the lack of understanding of the basic events underlying microaggregate formation, because of the availability of ultra filters which effectively remove microaggregates from stored blood, and because of serious questions as to the pathological significance of microaggregates in massive transfusion, no real attempt has been made to influence the development of microaggregates in stored blood. Should prevention of microaggregate formation prove clinically important, a better understanding of the underlying mechanism of microaggregate formation will be essential to institute effective preventive measures.

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PLASTICIZERS IN STORED BLOOD

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Plasticizers and microaggregates as blood contaminants are similar in that their concentrations increase throughout storage, they are unintentionally infused into recipients, and their exact toxicological hazard is yet undefined. However, not only is the chemical composition of plasticizer contaminants (phthalate esters) distinctly different from that of "microaggregates" (fibrin/platelets/"debris") but also there is a major difference in particle size. Blood may be freed of "microaggregates" by filtration through a 100μ filter. In contrast, phthalate esters, which are bound to plasma albumin and lipoprotein, easily pass through a 0.22μ filter.

Interest in the toxic potential of the principal plasticizer contaminant of blood storage, di-2-ethylhexyl-phthalate (DEHP), was kindled by reports of DEHP detection in tissues of patients receiving stored blood products (1). However, discovery of DEHP in tissues of humans who had not received transfusions (2), serious disagreement between research laboratories on results of assay determinations of DEHP in identical aliquots of DEHP-spiked tissue samples (3), and frequent assay contamination with ubiquitous environmental DEHP (4), all have made reports of DEHP detection in human tissues difficult to interpret.

Potential DEHP toxicity has been inferred from numerous animal studies. However, most studies of animal DEHP toxicity have described routes of administration, dosage formulations, and dosages which do not simulate the exposure pattern in man (5-7). Correspondingly, the relevance of such studies to human parenteral exposure to relatively low doses of protein-bound DEHP in stored blood products is not amenable to scientific evaluation at present. Two exceptions to this criticism bear mention. Garvin *et al.* (8) reported that no acute or cumulative toxicity could be demonstrated in rats infused twice weekly for 9 weeks with DEHP-rich stored homologous plasma. Each infusion resulted in a DEHP dosage equivalent to that which a human recipient would receive from a 12-unit whole blood transfusion.

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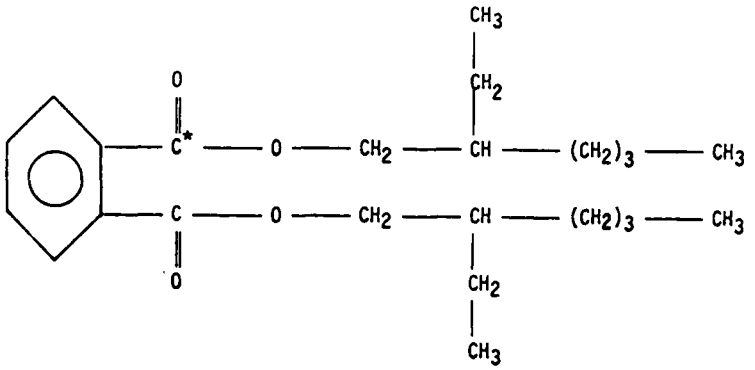
On the other hand, Jacobson and coworkers (9) have recently reported "DEHP" hepatotoxicity in rhesus monkeys given weekly autologous infusions for 1 year of DEHP-rich platelet concentrates which had been stored at room temperature for 72 hr. The DEHP infused each week was roughly equivalent to that a human would receive from a 2-unit whole blood transfusion. Whether these investigators correctly interpreted their results as "DEHP" toxicity is uncertain since the exposure levels of mono-2-ethylhexyl-phthalate (MEHP) were not reported (see comments on MEHP under "DEHP Leaching" below). The importance of paying careful attention to details of relative dosage, the physical state of the dosing formulation and composition in the planning and interpretation of DEHP toxicity studies has recently been emphasized (10).

Although human toxicity to DEHP through blood transfusions has not been documented, there remains a concern for the potential toxicity of this compound. In the absence of direct clinical toxicity data, the potential for various effects may be evaluated from knowledge of tissue distribution, the chemical nature of DEHP metabolites and conjugates, the organs involved in DEHP metabolism and elimination, and the time course of these phenomena. It is in this context that we review current knowledge on the disposition of DEHP in primates and man.

DEHP Leaching

The chemical structure of DEHP is shown below:



DEHP consists of a benzene ring to which are attached two carboxyl groups. Each of the latter is coupled to a 2-ethylhexanol side chain by an ester bond. The asterisk on the carbonyl carbon indicates the position of a ^{14}C label used in studies of the disposition of DEHP in the African Green Monkey. This carbonyl carbon appears to be the site at which plasma and tissue esterases in stored blood and in the body of primates act to cleave one of the two ethylhexanol side chains to produce MEHP.

DEHP is a clear oily liquid which is highly fat soluble and poorly water soluble. In polyvinylchloride (PVC) blood storage containers, the plasticizer resides in the PVC matrix as a semisolid and readily migrates from the plastic into plasma during storage. DEHP accumulates in plasma during 4°C liquid whole blood storage at a rate of approximately one milligram per unit per day and 12 milligrams per unit of platelet concentrate per day during storage at room temperature.

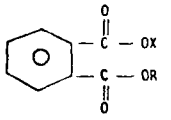
Recent studies done in our laboratories indicate that plasma DEHP undergoes hydrolysis to MEHP during storage, presumably under the influence of plasma nonspecific kipase. This reaction results in accumulation of MEHP in whole blood stored at 4°C (0.8-2.9 mg/unit after 21 days storage), as well as in platelet concentrates stored at room temperature (0.82 mg/platelet pack after 50 hr storage). The rate of this reaction appears to be different in bags from different manufacturers. Interest in MEHP stems from recent evidence indicating that large oral doses of MEHP are hepatotoxic in rodents (11).

African Green Monkey Studies

We began our investigation of the disposition of DEHP in primates by studying the distribution/elimination kinetics and metabolism of this compound in the African Green Monkey (12,13). In order to simulate closely the manner in which man is exposed to this compound, we procured a strip of polyvinylchloride plastic (Fenwal Laboratories, Chicago) which was impregnated with ^{14}C-DEHP. This sheet exhibited physical characteristics identical to that of Fenwal's PL-146 blood bag plastic. Twenty milliliters of plasma were harvested from each of three African Green Monkeys; PVC strips containing ^{14}C-DEHP were immersed in these plasmas and stored at 4°C for up to 5 months. At the time of reinfusion, these plasmas each contained around 3 mg of ^{14}C-DEHP - a dose equivalent to a 2 unit infusion of 21-day-old bank blood in a human. Thin-layer chromatography (TLC) and gas chromatography/mass spectrometry analysis (GC-MS) (16) of one of the incubated plasmas revealed the ^{14}C to be distributed as 96% ^{14}C-DEHP and 4% ^{14}C-MEHP, a pattern closely resembling that of unlabeled plasma phthalates stored in conventional PL-146 blood bags. Following bolus infusion of the ^{14}C-DEHP-laden autologous plasma, serial plasma, urine, and stool samples were obtained and counted for ^{14}C activity. As can be seen in Figure 1, plasma ^{14}C concentration rapidly declined so that by 90 min postinfusion there was less than 5%, and after 12 hr there was less than 1% of the initial concentration. Characterization of the chemical structure of the ^{14}C compounds in plasma during the first 30 min postinfusion revealed rapid conversion to MEHP and other oxidized

MEHP forms (Fig. 2). Figure 3 shows the buildup of ^{14}C in urine following the infusion.+ It can be appreciated that by 4 hr post-infusion, greater than 50% of the injected ^{14}C had been excreted in the urine, and that by 24 hr postinfusion, greater than 70% had been excreted in the urine. Table 1 reveals the chemical structure of DEHP metabolites in urine of the African Green Monkey (identified by GC-MS) (16); the predominant metabolites were the 5-ethyl, isohexanol mono ester of phthalic acid and MEHP. Greater than 80% of urinary metabolites were conjugated to glucuronide. The cumulative urinary excretion patterns of DEHP metabolites during the first 4 hr are plotted in Figure 4. Fecal ^{14}C excretion accounted for up to 8% of the injected ^{14}C by 48 hr postinfusion. The theoretical urinary excretion at infinite time (Fig. 3) coupled with the measured fecal excretion of ^{14}C accounted for the entire injected dose.

METABOLITE		% OF DOSE
CODE	R-STRUCTURE*	4 HOURS POST-INFUSION
12 B	CH_2-CH(C_2H_5)-(CH_2)$_2$-HC(OH)-CH_3	26.1
MEHP	CH_2-CH(C_2H_5)-(CH_2)$_3$-CH_3	19.6
Unhydrolyzed†	? ? ? ? ?	5.4
11	CH_2-CH(CH_2CH_2OH)-(CH_2)$_3$-CH_3	3.2
10	CH_2-CH(C_2H_5)-(CH_2)$_2$-CO-CH_3	3.1
11 X	CH_2-CH(C_2H_5)-(CH_2)-CHOH-CH_2-CH_3	2.7
9 E	CH_2-CH(C_2H_5)-(CH_2)$_3$-COOH	2.7
DEHP	X=R=CH_2-CH(C_2H_5)-(CH_2)$_3$-CH_3	<1.8
9 A	CH_2-CH(C_2H_5)-(CH_2)-COOH	trace
9 C	CH_2-CH(C_2H_5)-(CH_2)$_2$-COOH	trace
13 B	CH_2-CH(C_2H_5)-(CH_2)$_3$-CH_2OH	trace
Phthalic Acid H		trace
TOTAL		66.9

* 

X = glucuronide or H

†by β-glucuronidase or sulfatase

Table 1. DEHP metabolites in urine (African Green Monkey).

+The solid line running through the data points was obtained from the following equation: $U(t) = CL_r / \int_0^t Cpdt$; where U(t) = cumulative ^{14}C excretion, CL_r = renal ^{14}C clearance, C_p = plasma ^{14}C concentration = $Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$, the coefficients of which were obtained by fitting $C_p(t)$ to the C_p - time data.

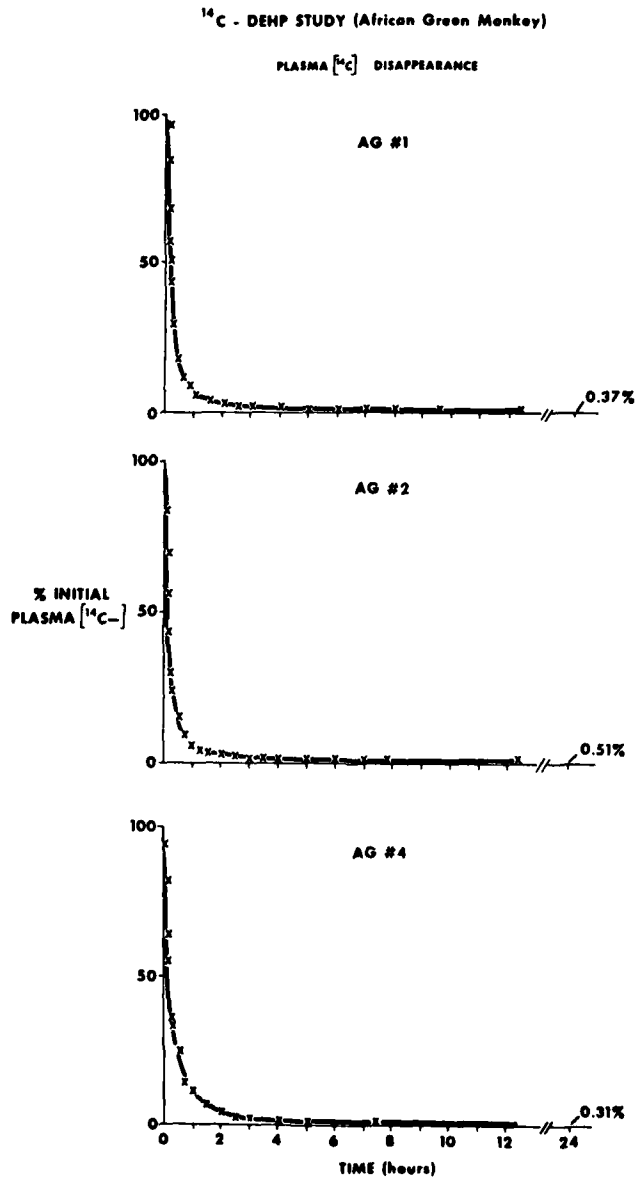


Fig. 1. Plasma ^{14}C concentrations in three African Green Monkeys following bolus injection of ^{14}C-DEHP leached into autologous plasma.

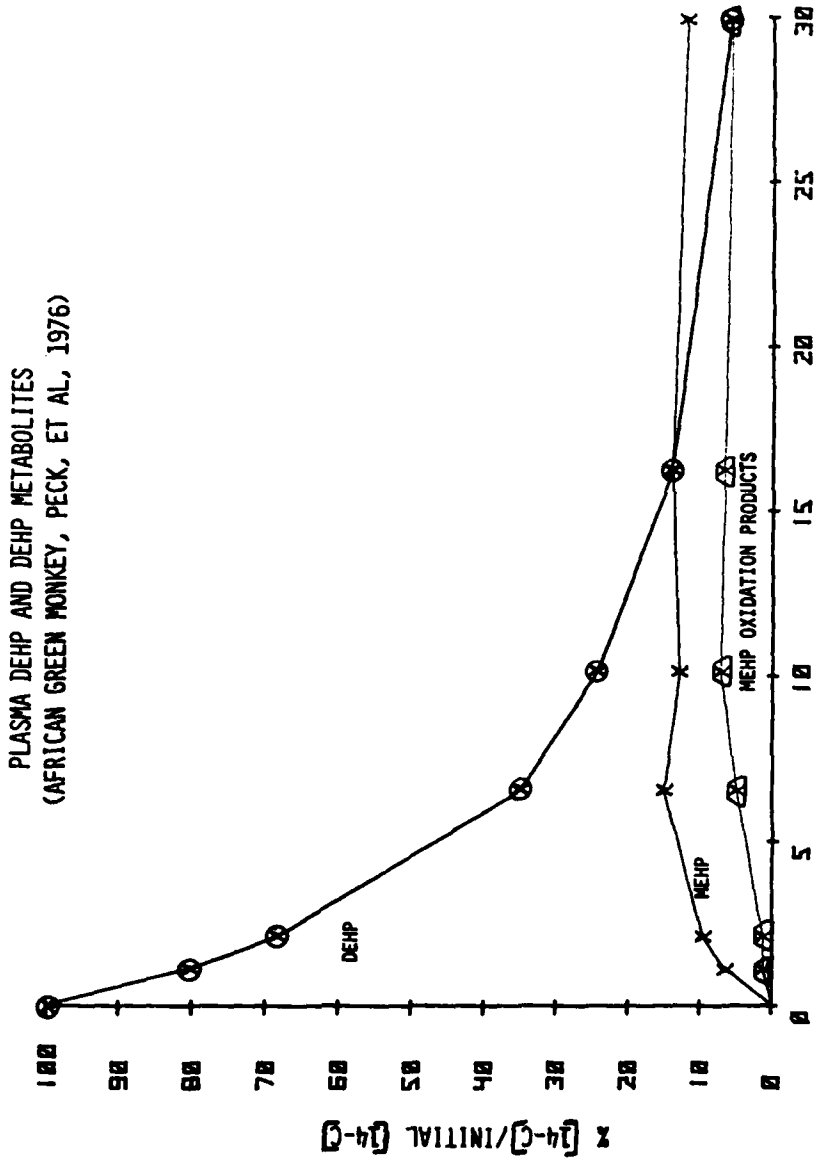


Fig. 2. Plasma DEHP and DEHP metabolites during 30 minutes following bolus injection of ^{14}C-DEHP leached into autologous monkey plasma (AG#4).

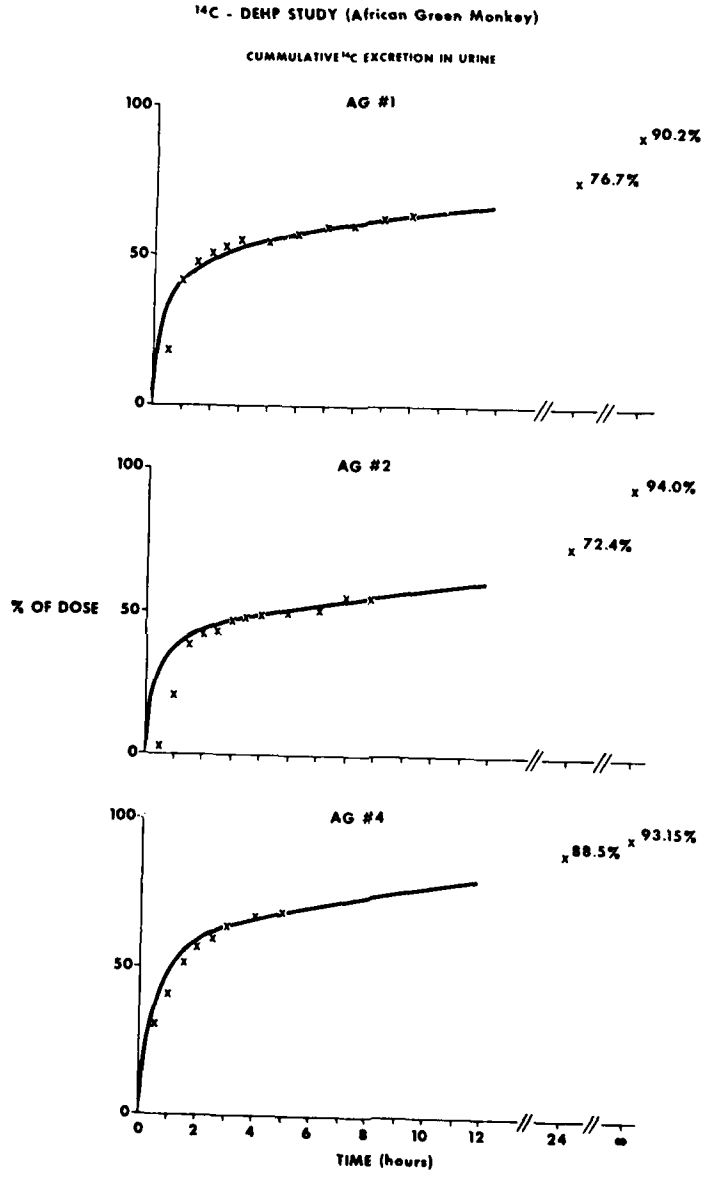


Fig. 3. Cumulative ^{14}C excretion in urine of three African Green monkeys following bolus injection of ^{14}C-DEHP leached into autologous plasma. See text for procedure used to draw solid line and estimates of cumulative excretion at after 24 hr and at infinite time.

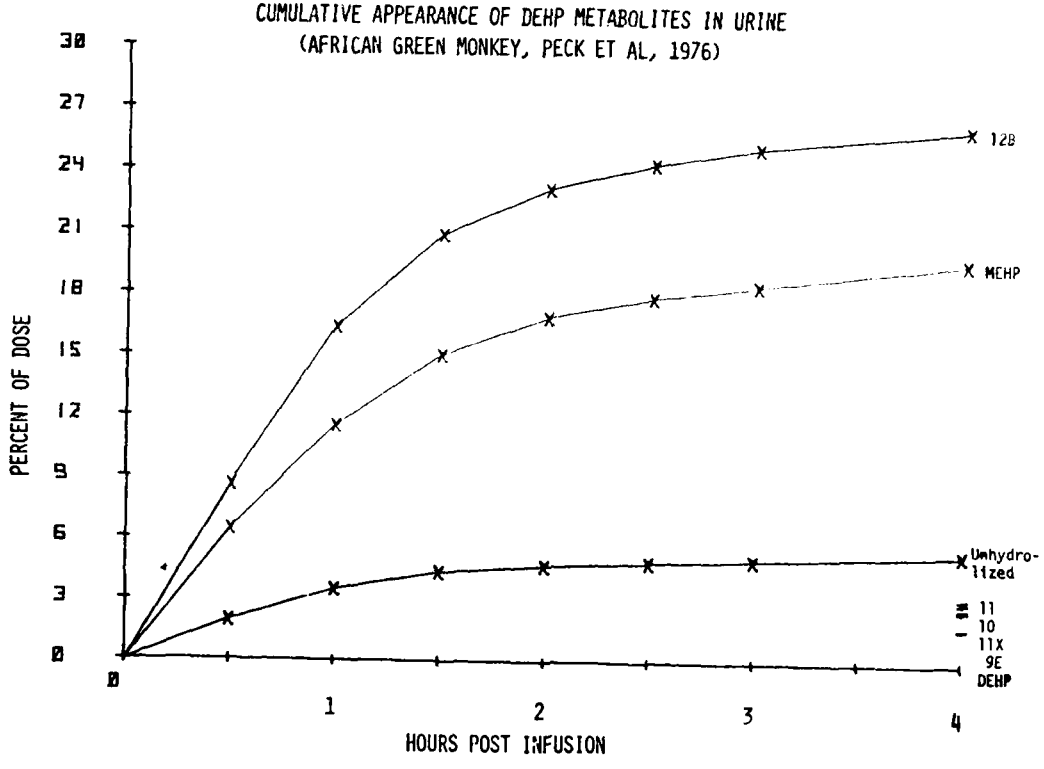


Fig. 4. Cumulative excretion of individual DEHP metabolites in urine of African Green monkey following bolus injection of ^{14}C-DEHP leached into autologous monkey plasma (AG #4). The metabolite codes are the same as those in Table 1.

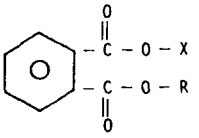
DEHP Disposition in Man

Recently, we had the opportunity to study the disposition of DEHP in two cancer patients receiving rather large doses of DEHP via platelet concentrate infusion. One patient (Case I) received 94.7 mg of DEHP in 4 hr, and the other (Case II) received 174.3 mg of DEHP in 1.5 hr. Figure 5 shows the cumulative urinary excretion of all DEHP derivatives. Greater than 50% of the infused dose appeared as DEHP derivatives in urine within 6 hr. This pattern is very similar to that seen in the African Green Monkey (cf. Fig 3). Table 2 details the chemical structure of the urinary metabolites (Case II) which were identified by GC-MS (16). As in the African Green Monkey, the predominant metabolite was the 5-ethyl, isohexanol mono ester of phthalic acid, but in contrast to the urinary metabolite pattern in the monkey, MEHP is fourth on the list and is followed by four more fully oxidized derivatives of MEHP. Again, approximately 80% of the urinary metabolites were conjugated to glucuronide. Figure 6 details the cumulative urinary concentration of various DEHP derivatives in Case II throughout the first 24 hr postinfusion.

DEHP METABOLITES IN HUMAN URINE

(CASE II)

METABOLITE		CUMULATIVE % OF DOSE
CODE	R-STRUCTURE	24 HOURS POST-INFUSION*
12-B	$-CH_2-CH(C_2H_5)-(CH_2)-HC(OH)-CH_3$	23.4
11	$-CH_2-CH(CH_2CH_2OH)-(CH_2)_3-CH_3$	8.3
10	$-CH_2-CH(C_2H_5)-(CH_2)_2-CO-CH_3$	7.7
MEHP	$-CH_2-CH(C_2H_5)-(CH_2)_3-CH_3$	6.9
11-X	$-CH_2-CH(C_2H_5)-CH_2-CHOH-CH_2-CH_3$	6.1
9-E	$-CH_2-CH(C_2H_5)-(CH_2)_3-COOH$	4.7
9-D	$-CH_2-CH(CH_2COOH)-(CH_2)_3-CH_3$	1.5
9-B	$-CH_2-CH(COOH)-(CH_2)_3-CH_3$	1.2
13-B	$-CH_2-CH(C_2H_5)-(CH_2)_3-CH_2OH$	trace
TOTAL		59.8†



X = glucuronide (80%) or H

*each is underestimated by <2% due to sample loss

†underestimated by ≈6.1%

Table 2. DEHP metabolites in human urine (Case II).

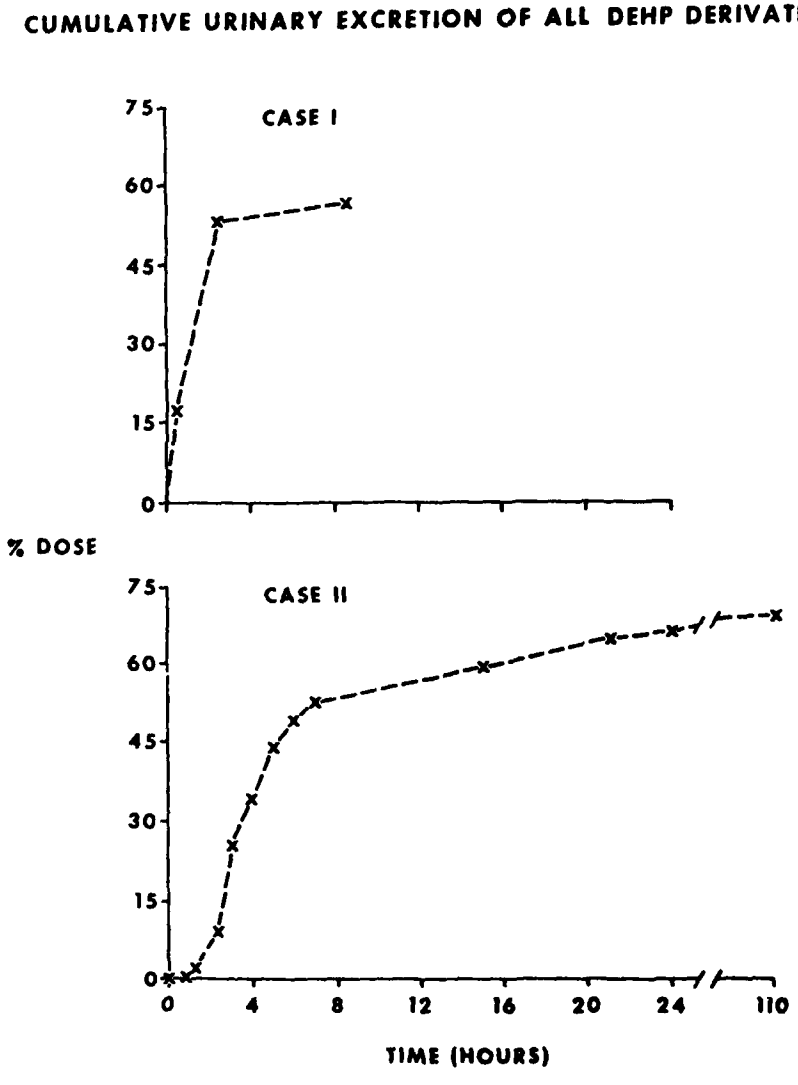


Fig. 5. Cumulative urinary excretion of total DEHP metabolites in two cancer patients following platelet concentrate infusions containing leached DEHP (Case I - 94.7 mg; Case II - 174.3 mg). After Peck, et al., 1978, with permission by Vox Sanguinis.

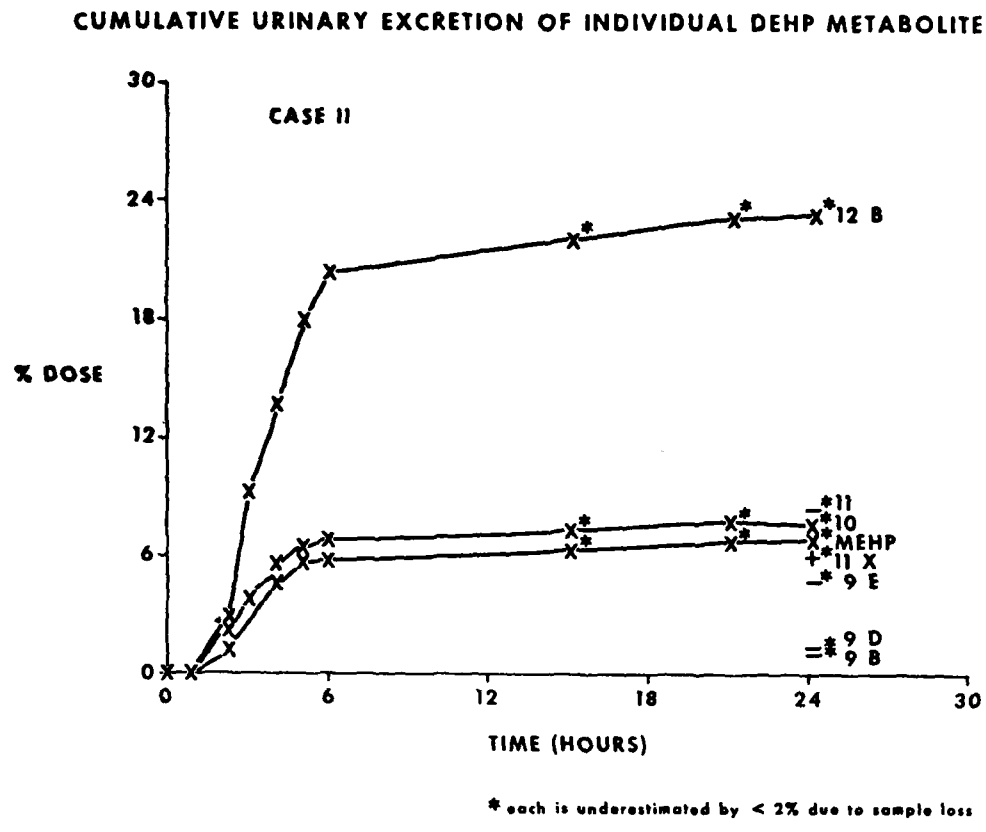


Fig. 6. Cumulative urinary excretion of individual DEHP derivatives in a cancer patient (Case II) following a 1.5 hr platelet concentrate infusion containing 174.3 mg of leached DEHP. The metabolite codes are the same as those in Table 2.

A recent study (14) enabled a pharmacokinetic analysis (15) of plasma DEHP disappearance in man. Plasma disappearance half-life was 30±12 min, apparent distribution volume was 2819±383 ml/M^2 and clearance was 78±20 ml/min/M^2 ($\underline{n}$=6). Based upon these pharmacokinetic parameters, Figure 7 shows the plasma DEHP/time profile for a simulated platelet infusion in which 90 mg of DEHP contained in 530 ml of pooled platelet concentrate are infused throughout a 30-min period into a 70-kg man. The DEHP is distributed in a volume which is slightly larger than blood volume, disappears rapidly from the plasma and is essentially gone from the plasma 4 hr after infusion.

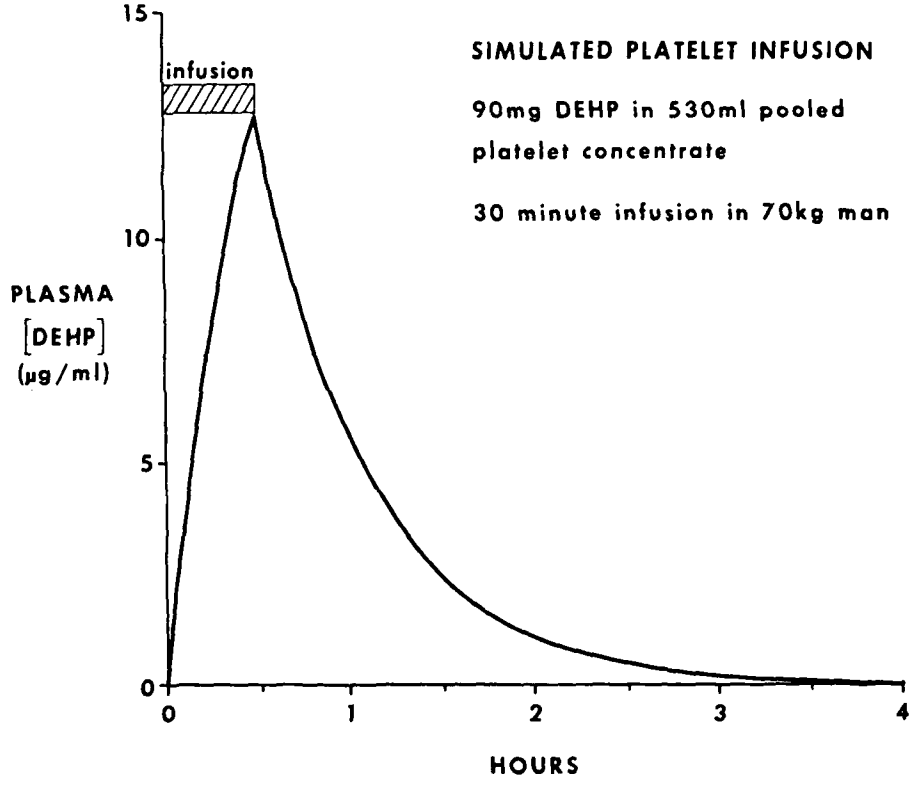


Fig. 7. Simulated plasma DEHP concentrations during and after 30-min infusion of 530 ml of pooled 72-h4-old platelet concentrate (90 mg DEHP total). The simulation is based upon average pharmacokinetic parameters derived (15) from six patients receiving DEHP-rich platelet concentrates (14), and assumes a body surface area of 1.73 M^2. With permission by Vox Sanguinis, 1978.

Discussion

Consideration of these kinetic and metabolic data in the light of potential toxicity in humans leads to some preliminary answers but many new questions. The restricted apparent distribution space, rapid plasma disappearance, rapid urinary excretion and extensive hydrolysis, oxidation, and glucuronide conjugation could all be interpreted as "detoxification mechanisms." Such an interpretation, however, requires knowledge of tissue sensitivities to DEHP and its metabolites. In the absence of such information, DEHP disposition data can assist our assessment of potential DEHP toxicity in several ways. For instance, the extensive metabolism and rapid excretion of DEHP metabolites cast serious doubt on the contention that DEHP found in human tissues is a result of blood transfusions; contamination during sample preparation and assay appears to be a more likely explanation. Moreover, the rapid excretion of the bulk of DEHP metabolites within 8 hr following infusion of blood products suggests that any toxicity from this source is more likely to be acute in onset rather than a late-appearing or long-term effect. Any toxicity observed may be the effect of a metabolic product of DEHP rather than the parent compound. In this regard, the recent discovery of MEHP accumulation during storage and report of its toxicity in rodents (11) should focus future research efforts on this compound in particular. Since the enzymes responsible for hydrolysis, oxidation, and conjugation of DEHP and MEHP may be located primarily in the liver, this organ, as well as the kidney, will be exposed to relatively high concentrations of DEHP derivatives. This knowledge should focus DEHP toxicity research on these two organs, as well as alert investigators to the possibility that toxicity may appear with altered DEHP metabolism or excretion due to hepatic or renal disease.

There is no evidence at present that humans who receive DEHP-laden blood products experience any adverse effects from DEHP exposure. Our studies of DEHP disposition demonstrate conversion to conjugated hydrolyzed oxidation products of MEHP which are then excreted in the urine. These metabolic processes account for elimination of greater than 50% of the infused DEHP during 8 hr postinfusion, while elimination of the entire dose is virtually complete in 4-5 days. Whether these metabolic events account for the lack of observed toxicity remains an unanswered question.

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MICROAGGREGATES AND EMBOLI IN AUTOTRANSFUSION

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Donald B. Doty, M.D., FACS,* Glenn W. Geelhoed, M.D.**

The salvage and return of an individual's own shed blood was initially used by Blundell in 1818 (41). Since then, blood defibrinated in the thoracic or abdominal cavity has been collected, filtered through muslin or gauze, and reinjected intravascularly. The autotransfusion technique (ATS), however, was clumsy and, although life-saving, fell into disuse with the development of satisfactory methods for homologous blood storage and transfusion (20, 41) (Table 1). In recent years, however, there has been an increasing awareness and concern over the complications of blood transfusion, in particular, hepatitis, transfusion reactions, allergic reactions, and the possible administration of particulate debris (18, 20). It has also been demonstrated that in the major cities, adequate amounts of blood are not always available for massive trauma cases. Therefore, with the availability of a variety of devices and further definition of the physiologic and pathophysiologic effects of autotransfusion, this procedure has, once again, achieved some measure of popularity. The primary indications for autotransfusion are listed in Tables 2 and 3. Thus, autotransfusion has applicability in both emergencies with exsanguinating hemorrhage and inadequate blood reserves and elective procedures with a large anticipated, or sudden intraoperative blood loss. In the past few years, many groups (3-6, 11, 12, 19, 21, 23, 24, 27, 29, 37, 39) have reported excellent results when autotransfusion has been used. Nevertheless, a variety of theoretical and real complications have been discussed and most are listed in Table 4 (1-9, 11-13, 15, 16, 27, 28, 37-39).

Table 1. Historical review on use of autotransfusion.

1818	Blundell	First use of autotransfusion.
1874	Highmore	First article advocating autotransfusion.
1900	Landsteiner	Definition of ABO blood types.
1917	Lockwood	First use of autotransfusion in U.S.
1917	Elmendorf	Use of autotransfusion for hemothorax.
1970	Klebanoff	Effective disposable commercially available autotransfusion system.

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Table 2. Indications for autotransfusion.

1. Trauma with exsanguinating hemorrhage.
2. Extensive surgery with anticipated blood loss.
3. Inability to obtain stored blood.
4. Unusual blood type or failure of cross-match.
5. Bleeding diathesis (DIC).
6. Religious beliefs contraindicating standard transfusion.

Table 3. Elective uses of autotransfusion.

Vascular - portacaval shunting
  Aortic reconstruction
  Aortocaval fistula
  Reoperative vascular surgery
  Abdominal aortic aneurysm or
    thoracо-abdominal aneurysm
  AV malformations
Liver resections
Spleen removal of hypersplenism
Orthopaedic long bone reconstruction
  (spine fusions)

Table 4. Potential complications of autotransfusion.

1. Red blood cell destruction.
   - Hemoglobinemia
   - Hemoglobinuria
2. Bacteremia - septicemia.
3. Coagulopathy, diffuse intravascular or extravascular.
4. Embolization.
   - Air
   - Coagulum
   - Fat
   - Neoplasm
   - Microaggregated platelets and white cells
5. Pulmonary insufficiency.

Of these, red cell destruction is an integral and inescapable part of all extracorporeal circulation systems. In autotransfusion, the red cell destruction can be minimized by modifications of the suction apparatus and technique, particularly by reducing the negative pressure, by reducing the blood-air interface, and by suctioning primarily from a pool of blood rather than skimming the surface (1, 39). The reported levels (50-600 mg %) of hemoglobin

have been less than those encountered from hemolysis during cardiopulmonary bypass and are of shorter duration. Survival of returned red blood cells is comparable to that of normal blood (18, 37). The effects of the hemoglobinemia can be minimized by attention to adequate urine output and alkalinization of the urine.

The problem of bacteremia and septicemia is not a facet of the elective use of autotransfusion, but rather arises in cases of penetrating or blunt trauma to the abdomen where hollow viscera are disrupted. Interestingly, although bacteremias are clearly produced and demonstrated by gram stain, or obvious because of gross fecal spillage, the incidence of sepsis or migratory abscesses has been low (23). Presumably this is the result of maintenance of adequate white cell and reticuloendothelial systems, as well as the preservation of adequate complement and immune globulins.

Coagulopathies may occur following the intravascular return of partially clotted blood and fibrin split products, or from depletion of labile clotting factors and platelets. The appropriate use of local, regional, or systemic anticoagulants has been shown to reduce the incidence of bleeding diathesis (3). Nevertheless, in most series, an occasional patient will have persistent bleeding. This usually is in patients such as those with abdominal aneurysm rupture, when postoperative bleeding is common even when ATS is not utilized. Platelets are reduced during extracorporeal circulation and autotransfusion compared to the patients' normal fresh blood, but are clearly more numerous than in the usual homologous stored blood.

Macrodebris of all sorts may be introduced into an extracorporeal system. Others have reported finding bubbles, fragments of bone, lobules of fat, dried or clotted blood, tumor cells, or other tissue fragments (7, 9, 10, 14, 25, 44). In part, attention to detail in limiting the area and pool of collection for autotransfusion will eliminate the greatest mass of this debris (11). The remainder should be removed by the primary filtration system in the collection chamber. Depending upon the type of system utilized, the risk of venous air embolization can also be alleviated by attention to the reinfusion apparatus and the presence either of bubble traps, warning signals, or allowing the reentry to occur under gravity rather than pressure infusion. Some current devices allow blood collection into closed air-free containers and thus are not subject to air embolism even if pressurized (21, 39, 40).

The present symposium is dedicated to a discussion, however, of microaggregates in various circulations.

The presence of microaggregates has been documented by techniques such as direct microscopy, repetitive platelet counts, screen filtration pressure measurement (SFP), ultrasound, and Coulter counter

particulate volume analysis (8, 17, 25, 26, 30-36). Aggregates of platelets may occur in stored blood, hypovolemia, endotoxin shock, stress, and spontaneously, as well as be induced by a variety of drugs or procedures (8-10) (Table 5). We, as well as others, have evaluated the occurrence and removal of microaggregates of platelets in canine, subhuman primate, and humans during autotransfusion (18). In 2-4 blood-volume-autotransfusions in normotensive heparinized (300 u/kg) dogs with a groin incision used as a collection pocket, Rakower (3) demonstrated platelet clumps microscopically in blood taken from the pocket. The SFP was not regularly elevated. Blood collected without tissue contact had no clumping of platelets. Necropsy demonstrated only minimal and variable congestive changes in the lungs.

Table 5. Conditions that may cause microaggregates.

Stored blood.
- Hard aggregates
- No useful platelets
- Filterable
- Proven tissue damage

Extracorporeal aggregates.
- "Fresh-soft" aggregate
- Functional platelets
- Filterable
- Tissue damage controversial

Bennett and associates (1) also studied massive autotransfusion (4-12 blood volumes) in heparinized (200 u/kg) dogs bled into the abdominal cavity. Blood pressure was not reported. The SFP measurements on the peritoneal blood were immeasurably high in 16 of 20 animals and elevated in all 20. Macro- and microfiltration reduced SFP to 145 $\pm$ 11 and 38 $\pm$ mm Hg, respectively. Platelet counts were decreased (280,000 to 180,000) but not to a level which was likely to cause spontaneous bleeding. Hemodilution and the idiosyncratic thrombocytopenic response of dogs to heparin may play a role in the lowered platelet counts. All the animals survived without functional organ impairment. Subsequent lung perfusion studies in the same laboratory using autotransfusion, fresh, and stored blood showed no increase in pulmonary resistance or endobronchial pressure with autotransfused blood (2).

Wright and Solis (42) studied microaggregation and filtration in heparinized (300 u/kg) dogs made hypotensive during the procedure (Table 6). Large microaggregates of platelets occurred and could be removed by filtration. The effectiveness of the filters available at that time was assessed in this and other studies in the same lab (18, 26, 30-32). Spontaneous deaggregation of these platelet clumps was demonstrated (Fig 1) over a short interval. Organ damage was not assessed in this study. The functional capacity of the retrieved platelets was not measured but Solis *et al.* (33) have

suggested that platelet function is good in platelets studied during extracorporeal bypass.

Table 6. Autotransfusion samples.[1]

Sample Source	Particle Volume (μ^3 X $10^3/mm^3$)
Normal arterial sample	91 ± 18
Autotransfusion reservoir (immediate)	1,740 ± 385
Autotransfusion reservoir (after 1 hr)	270 ± 76
Bentley 120 μ mesh (Cardiotomy Reservoir)	1,236 ± 324
Barrier 40 μ mesh (Johnson & Johnson)	883 ± 198
Pioneer Dacron wool (Swank)	200 ± 30

[1]Wright, C. B. and Solis, R. T. (42), with permission by American Journal of Surgery.

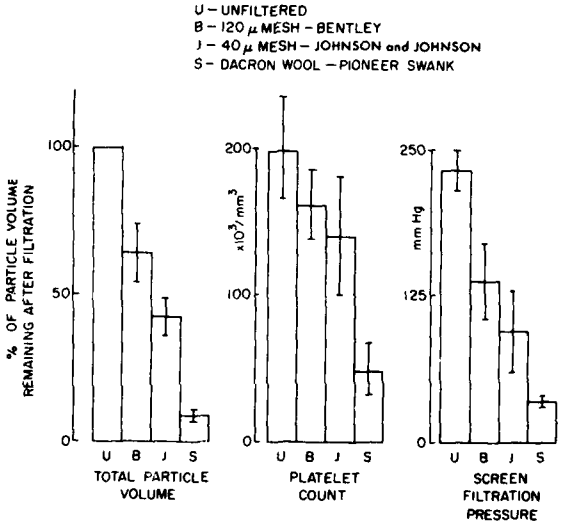

Fig. 1. Percent change in cumulative volume of particles remaining after filtration, platelet counts, and screen filtration pressures are expressed as the mean ± SE of nine experiments in nine dogs. Wright, C. B. and Solis, R. T., (42), with permission by American Journal of Surgery.

In summary, the dog experiments suggest: 1) autotransfusion can be done safely with restoration of blood volume and acceptable hemolysis levels. 2) Microaggregation of platelets occurs during tissue contact and hypotension but minimally, or not at all, in heparinized, normotensive, extracorporeal circuits without peritoneal or other tissue contact. 3) Microaggregates can be effectively removed by filtration. 4) The in vivo functional significance of these particles which deaggregate promptly in vitro is not certain. (See discussion in this symposium by George P. Claggett, Maj, MC.)

Using the Anubis baboon, an animal phylogeneticallv closer to man, Wright and coworkers (43) performed large volume (4 x blood volume) autotransfusion with systemic heparinization (300 u/kg) and careful maintenance of normotension (Fig. 2 and 3). An inguinal incision served as the collection pocket. Platelet counts during the autotransfusion were transiently, but insignificantly, reduced and remained normal 48 hr after the procedure (Fig. 4). No spontaneous bleeding occurred in any of the animals and they all survived. It was observed that SFP and Coulter particle volume measurements were not increased during these autotransfusions (Fig. 5). This is in contrast to demonstrated microaggregation in our laboratory and by others in heparinized dogs subjected to autotransfusion during some degree of hypotension. I believe the primate data support the position that the autotransfusion apparatus and the procedure of autotransfusion do not necessarily produce microaggregation or microembolization and do not lead to pulmonary function deterioration (Fig. 3).

Brener and associates (6), using autotransfusion during elective vascular surgery with heparinization (100-300 u/kg) have observed hemolysis (50-600 mg% hemoglobin), but no significant renal complications, and no coagulopathies. SFP measurements were increased significantly (90-400 mm Hg) but DacronR wool microfiltration returned this value to normal (50-60 mm Hg). Platelet counts were not significantly reduced. No emboli or embolic sequellae were identified postoperatively.

Wall et al. (39) have reported a series of patients who had vascular surgery with autotransfusion and no use of homologous blood in five of six. Heparin, 300 u/kg, was used during collection and protamine was subsequently used to achieve reversal. A microfilter was used on the infusion circuit. Hemoglobinemia was variable (20-840 mg %) but produced no significant complications. No bleeding diathesis occurred. Platelets were not significantly reduced. The patients had no evidence of embolic phenomena.

These and other data accumulated under controlled circumstances in normotensive, heparinized man with moderate autotransfusion parallel the data collected from animal experiments, (5, 6, 39) and support the contention that the risks and complications of autotransfusion are minimal and the potential benefit great. The

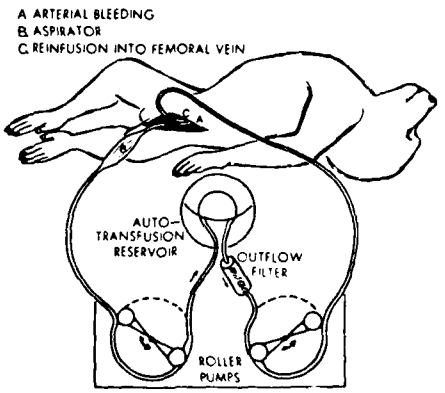

Fig. 2. Diagrammatic presentation of the experimental preparation in baboons. Wright, C. B. et al. (43), with permission by American Journal of Surgery.

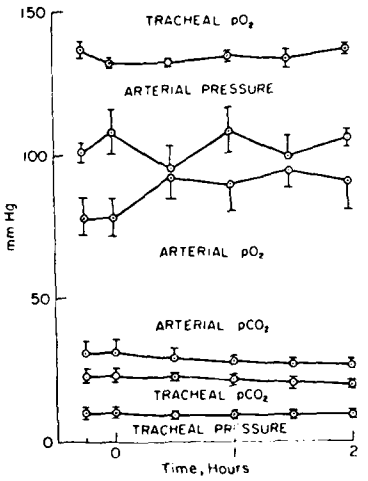

Fig. 3. The effects of two hours of autotransfusion on cardiorespiratory parameters are presented. Each point represents the mean ± the standard error of five experiments in five baboons. Wright, (43), with permission by American Journal of Surgery.

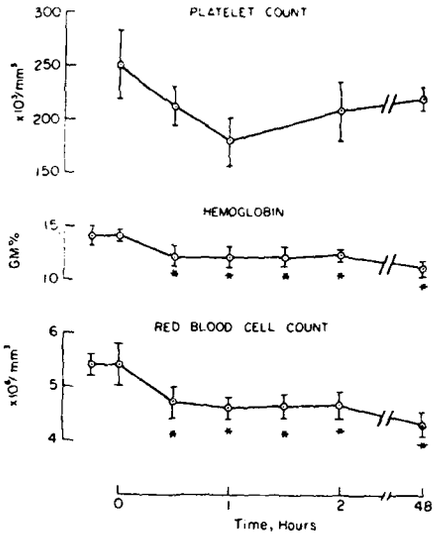

Fig. 4. The effects of two hours of autotransfusion on platelet count, hemoglobin, and red blood cell count are presented. Each point represents the mean ± the standard error of five experiments. The asterisk indicates that the value is significantly different from the control ($p<.05$). Wright, C.B., et al. (43) with permission by American Journal of Surgery.

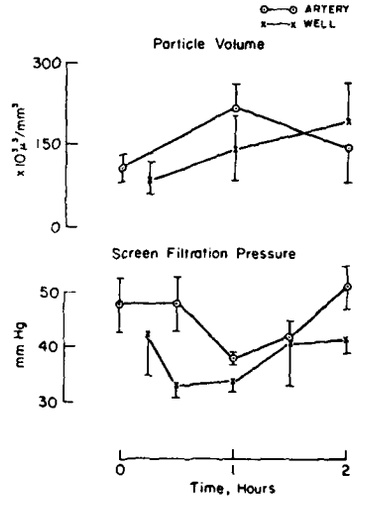

Fig. 5. The effects of two hours of autotransfusion on particle volume and screen filtration pressure are presented. Each point represents the mean ± the standard error of five experiments in five animals. Wright, C.B., et al. (43) with permission by American Journal of Surgery.

growing experience with use of autotransfusion in trauma has salvaged many patients, but the complications of the trauma have occasionally been blamed on autotransfusion systems.

Clinically then, although some individuals following massive trauma and autotransfusion may have renal insufficiency, bleeding, or pulmonary insufficiency, these complications would appear to result from their primary conditions rather than the procedure of autotransfusion (1, 5, 11, 12).

In conclusion, from a review of the literature and our own experimental evidence, it appears clear that microdebris of a variety of sorts may be created and introduced during autotransfusion and extracorporeal circulation. Effective filtration of stored blood, filtration of blood collected through suction apparatuses to eliminate all gross debris, and the use of auxillary filters (1, 2, 8) to eliminate microparticles seem desirable during clinical autotransfusion. For this reason, for both emergency and elective autotransfusion, a specific system and supervisor have been established in our institutions (Table 7).

Table 7. Method for autotransfusion.

Protamine reversal of heparin (ACT).
Systemic heparinization - 300 u/kg (elective) or systemic heparin monitored by activated clotting time (ACT) during the autotransfusion.
Large intravenous lines.
Saline prime of the system.
Auxillary filtration (Dacron wool microfilter).
Sump suctioning of the blood to be returned.
Blood return, 1) from blood bags, or 2) direct pressure.
Supervisory person present.
Attention to diuresis and plasma - blood pH.

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MICROAGGREGATES IN AN ISOLATED LUNG PERFUSION MODEL

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Microaggregation of cellular and subcellular particles in blood has been one factor implicated in the changes in pulmonary form and function in lungs perfused by blood containing these microaggregates. The pulmonary capillary bed is the first filter in the body for anything infused intravenously, and the inference has been that any substance infused on the right side of the heart that would contain or cause microaggregation in blood would result in pulmonary microemboli.

Pulmonary insufficiency is one of the life-limiting complications that may follow severe trauma or massive operative injury. Factors common to these injuries and their treatment methods include shock and massive transfusion of blood and infusions of fluids intravenously. Microaggregates have been demonstrated by elevated screen filtration pressures in banked blood and in the injured patients receiving it (1). A rational inference from this clinical information is that the microaggregation demonstrable in the infused stored blood may be the antecedent cause for the posttraumatic pulmonary insufficiency.

The cellular and particulate microemboli that reach the lungs might cause injury by several mechanisms that have been proposed. The aggregated particles themselves can cause pulmonary vascular obstruction and a rise in pulmonary vascular resistance if they wedge in the pulmonary capillary bed which filters these aggregates out of further circulation. Or, additionally, cellular aggregates such as clumped platelets and some types of white cells might release vasoactive amines which may cause vasospasm in the pulmonary perfusion bed or evoke an inflammatory response with cellular infiltration cuffing pulmonary vessels, either or both of which would contribute to pulmonary vascular obstruction. Through each of the proposed mechanisms, damage results to the capillary and alveolar membranes with interstitial congestion and transudation of fluid into the air space decreasing the compliance and efficiency of the lung in respiratory gas exchange. These mechanisms, postulated to explain the clinical "shock lung" syndrome, are valuable since they permit experimental testing of the microaggregate hypothesis in a controlled laboratory model. One such model has been developed to test the microaggregation/microembolization hypothesis using an *in situ* canine and primate lung perfusion model.

MATERIALS AND METHODS

Animal Species and Blood Processing

Autologous blood was studied in perfusion of lungs in two species. Twenty-eight Papio species adult male baboons were used in the primate experiments and 21 adult mongrel dogs averaging 21 kg in size were used in the canine experiments. From each animal 500 ml of autologous blood were drawn into a standard acid citrate dextrose (ACD) blood recipient bag and stored at 4°C in the blood bank. After a period of blood storage which varied with the protocol for perfusion, each unit of blood was proven to be sterile by culture and each unit was passed through a standard commercial blood administration set filter (170 μ) prior to use in pulmonary perfusion.

The dogs were divided randomly into three groups; blood from the first group was stored for 24 hr ("fresh" blood), from the second group, 21 days ("old" blood), and from the third group the 21-day-stored old blood was filtered through a Dacron-wool (10 μ) filter after the blood had been passed through the standard blood administration set filter. The primates were divided into four groups. In the first three groups the blood was similarly "fresh" (24 hr), "old" (21 days) and Dacron-wool filtered old blood. In the fourth group the autologous blood was reconstituted from 24-hr-stored cells and 21-day-stored plasma after each had passed through the recipient set filter.

Additional experiments were carried out with autotransfusion blood in the primate (2), in the canine with and without Dacron-wool filtration (3), and the results of these experiments were reviewed elsewhere in this monograph (4).

Hematologic Measurements

In all instances the degree of microaggregation in both species was estimated by screen filtration pressure (SFP) determinations using the method and device of Swank (5). In addition, hematocrit, white blood cell counts, platelet counts, total protein, albumin and calcium determinations were made on the test blood before and at intervals during perfusion of the lung.

Animal Operation and Monitoring

Each dog was anesthetized with sodium pentobarbital and each baboon was given sernylan. A Carlen's endotracheal tube was advanced through the oral pharynx in the primates and by cervical tracheostomy in the dogs to a position where the right

and left lungs were ventilated independently with room air by means of synchronous volume respirators independently adjusted for a full tidal volume according to endobronchial pressure.

A left thoracotomy was performed in each animal and the left upper lobe of the lung removed. A large cannula was advanced through the heart into the left pulmonary vein after being introduced through the left atrial appendage. One hundred-fifty milliliters of blood were drawn through this cannula to prime the perfusion circuit and then the left pulmonary artery was cannulated directly completing the isolation of the left lower lobe in the perfusion circuit. The isolated lung remained in situ within the intact animal with innervation and lymphatic drainage of the left lower lobe undisturbed.

Pressure transducers and recorders were used to monitor pressures in the bronchus, pulmonary artery and pulmonary vein. Gas tensions in the pulmonary artery and vein were continuously monitored through silastic probes in the pulmonary arterial and venous catheters connected with a mass spectrometer which recorded pO2 and pCO2 in the pulmonary artery and vein.

Pulmonary Lobe Perfusion Protocols

The perfusion circuit for the left lower lobe in these animal models is schematically represented in Figure 1. Blood drained by gravity from the isolated pulmonary vein into a reservoir suspended from a strain-guage scale. The height of the reservoir was continuously adjusted to keep pulmonary venous pressure (Ppv) zero. The blood was advanced by a roller pump through a membrane lung deoxygenator in which it was purged of oxygen by a gas mixture of 85% nitrogen and 15% carbon dioxide. The blood arterialized in the animal's lung was thus desaturated in the membrane lung which was used as a substitute for the systemic capillary bed. The blood passed from the membrane lung with pO2 and pCO2 tensions compatible with systemic mixed venous blood. This desaturated blood passed through a bubble trap and heat exchanger and was pulsed through the pulmonary artery at a delivery rate preset by the output of the roller pump.

After baseline data were obtained during perfusion of the left lower lobe in each animal for 1 hr, the venous reservoir was replaced by the unit of autologous blood that had been stored for variable periods and filtered or processed according to the protocol of blood storage previously described.

Perfusion resumed with the unit of test blood mixing with the amount of heparinized fresh blood remaining in the tubing of that which had primed the perfusion circuit. This represented a 3:1 mixture of the test blood, diluting it with the fresh

priming blood. At 30-min intervals blood samples were drawn, and pressure/flow curves were determined by varying the output of the roller pump from 100 to 600 ml/min while Ppv was maintained at zero. The duration of perfusion with the test blood was 2 hr; however, in most instances in which aged blood was used, the lung degenerated before the end of 2 hr and data were recorded for the "end of perfusion" if that point was reached before 2 hr of the test blood perfusion. Lung biopsy specimens were taken of the inflated lungs at nondependent portions of the lung before and after perfusion with the test blood. These biopsies were weighed before and after desiccation to determine the lung water index, and were examined on histologic section and graded by an independent observer for the presence or absence of morphologic changes.

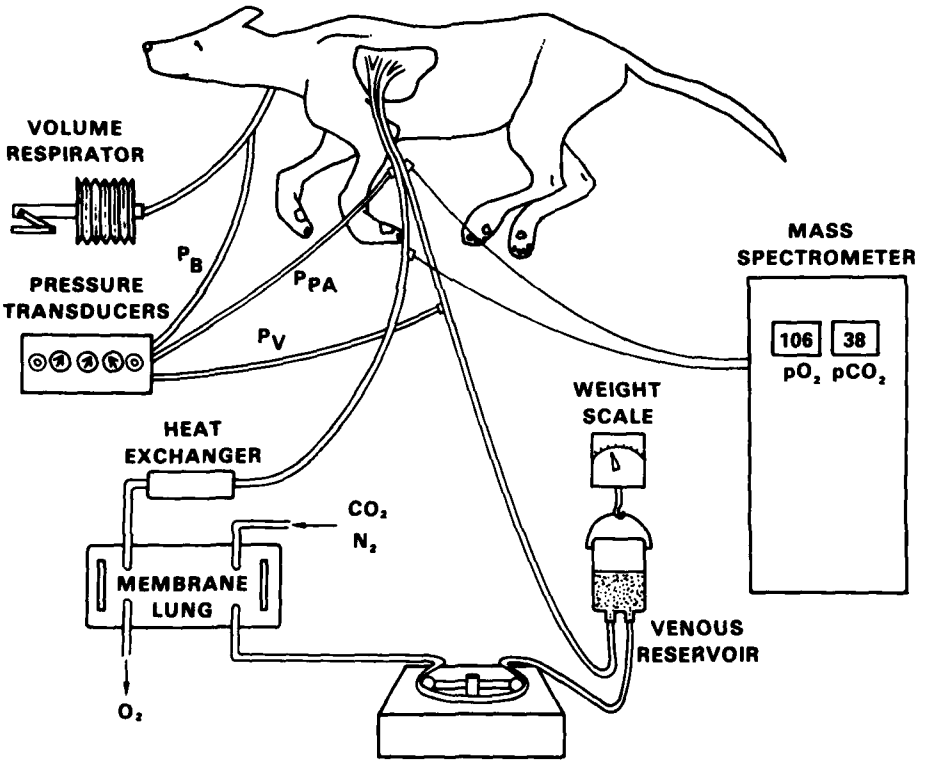

Fig. 1. The in situ pulmonary lobe perfusion model in schematic diagram representing the perfusion circuit and the equipment used to monitor changes in pressures and gas exchange.

RESULTS

The data obtained during perfusion in each of the animals studies were analyzed for changes seen in the aggregation of the blood as determined by SFP, and the physiology and morphology of the lungs perfused by the blood containing the variable microaggregate quantities.

Microaggregation in Stored Blood

The baboon blood stored in acid citrate dextrose (ACD) solution appeared to show similar changes as those reported for human blood during storage, in that a gradual elevation of SFP was noted during prolonged storage (Fig. 2). Blood storage in the dog, however, showed no significant change or consistent trend in SFP with prolonged storage in ACD solution (Fig. 2).

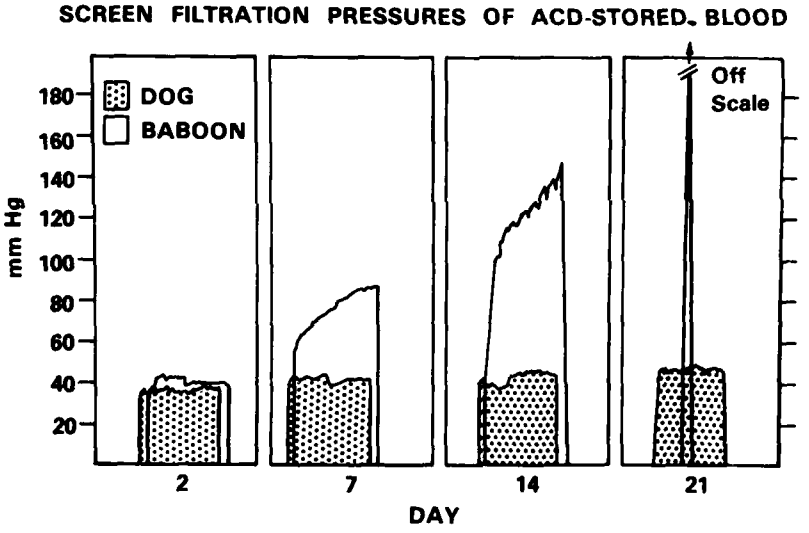

Fig. 2. Screen filtration pressures of primate and canine blood as a function of time.

Dacron-wool filtration did not significantly change the SFP of 21-day-stored canine blood which was not significantly different than fresh canine blood before filtration. In the baboon, Dacron-wool filtration reduced the SFP toward normal. Resuspending fresh cells in plasma from old blood raised the SFP over twice the value seen in fresh blood in the primate (Table 1).

Physiologic Changes During Perfusion

Changes were observed in the primate and canine experiments when the test blood was compared with the control period of

TABLE 1. Changes in screen filtration pressures and physiologic changes in lungs perfused with fresh, old, old-filtered, and fresh cells/old plasma (ACD-stored autologous blood).

Blood	Perfusion blood screen filtration pressure, mm Hg	Pulmonary vascular resistance, %	Effective compliance %	Aterio-venous PO_2 gradient, %	Wet/dry lung wt, %	Pulmonary edema[1]
Baboon						
Fresh (24 hr) blood	36	5 ↑	10 ↓	19 ↓	24 ↑	0/7
Old (21 day) blood	>200	129 ↑	74 ↓	45 ↓	43 ↑	6/7
Dacron-wool filtered old blood	54	21 ↑	8 ↓	27 ↓	30 ↑	4/7
Fresh cells/ old plasma	88	38 ↑	20 ↓	28 ↓	33 ↑	1/6
Dog						
Fresh (24 hr) blood	3.0	0.4 ↑	17 ↓	11 ↓	36 ↑	0/7
Stored (21 day) blood	1.4	70 ↑*	75 ↓*	60 ↓*	53 ↑*	6/7
Dacron-wool filtered stored (21 day) blood	3.1	33 ↑*	65 ↓*	38 ↓*	51 ↑*	5/7

[1]Number of animals showing gross or microscopic evidence.

perfusion in pulmonary artery pressures, endobronchial pressures, arterio-venous pO_2 gradient, lung fluid sequestration, and frothy edema fluid appearing in the airway. The changes in pulmonary artery pressure are expressed in units of pulmonary vascular resistance - a value derived by dividing the observed pulmonary artery pressure by the pulmonary blood flow, equal to the flow rate delivered by the perfusion pump. Changes in endobronchial pressure are translated to changes in effective compliance by dividing the difference in end-inspiratory and end-expiratory endobronchial pressure by the known constant tidal volume delivered by the independent left lung ventilator. The efficiency of the animal's perfused lung in effecting gas exchange was expressed for oxygen as the arterio-venous oxygen gradient; there was no significant change in pCO_2. Wet/dry lung weight changes reflect an increase in the lung water index expressed in grams of water per gram of dry lung tissue. Gross pulmonary edema was judged to be present when endobronchial froth appeared in the ventilator from the left lower lobe.

A summary of the physiologic changes seen in the primate and canine test blood perfusions is displayed in Table 1. The functional damage to the lungs in both species was most marked with old blood perfusion. Dacron-wool filtration decreased screen filtration pressures only in the primate species, but filtration did not render old blood innocuous to the lung in either primate or canine lung perfusion. At least some pulmonary injury resulted from perfusion of primate lungs with plasma from stored blood added to fresh cells. The amount of physiologic injury seen is midway between that shown by primate lungs perfused with fresh and old blood. Lung weight and gross evidence of pulmonary edema increased in both species in the animals perfused with old blood, whether or not filtered.

Morphologic Changes

Comparison of lung biopsies taken before and after perfusion with the test blood was done by scoring the histologic slides for the microscopic presence or absence of interstitial edema, perivascular hemorrhage, alveolar hemorrhage, intraalveolar fluid and congestion of alveolar septae. As seen in Figure 3a, few changes are seen after perfusion with old blood (Fig. 3b). Figure 4 shows similar changes in the canine lung after perfusion with fresh blood (Fig. 4a) in contrast with the appearance of the lung after perfusion with old blood (Fig. 4b).

Table 2 summarizes the morphologic changes seen in the primate and canine lungs. No significant changes were seen by any of the criteria applied to lungs perfused with fresh blood.

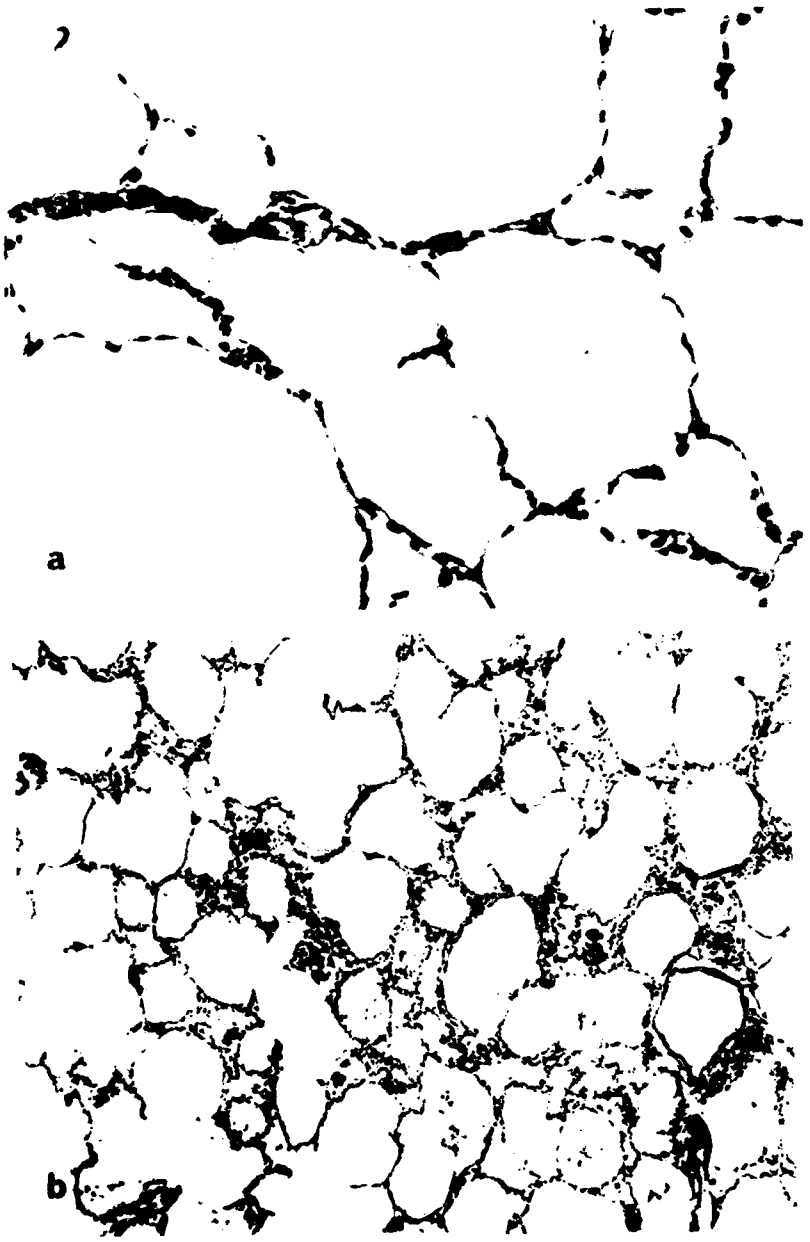

Fig. 3. Pulmonary morphologic changes compared in primate lung after perfusion with fresh (a) and old (b) blood; the morphologic changes were not significantly different in primate lungs perfused by Dacrol-filtered old blood or old plasma/fresh cells (a: hematoxylin-eosin X 250; b: hematoxylin-eosin X 63).

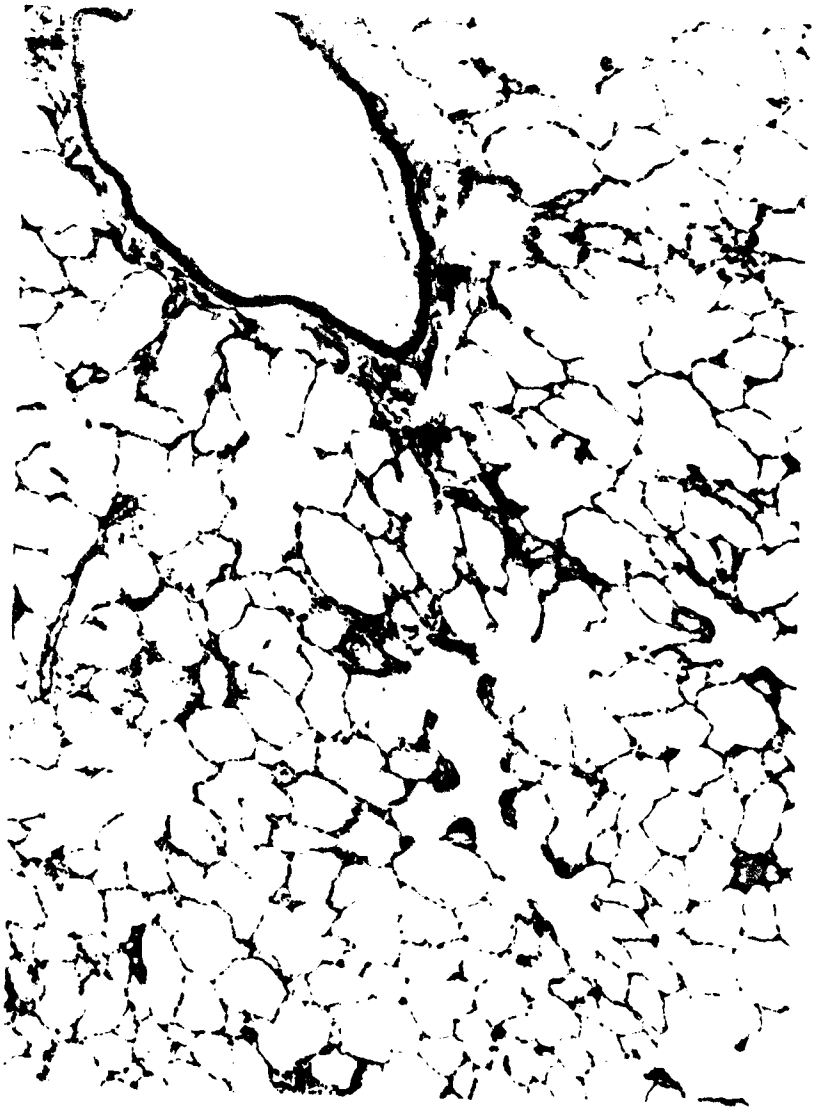

Figure 4a

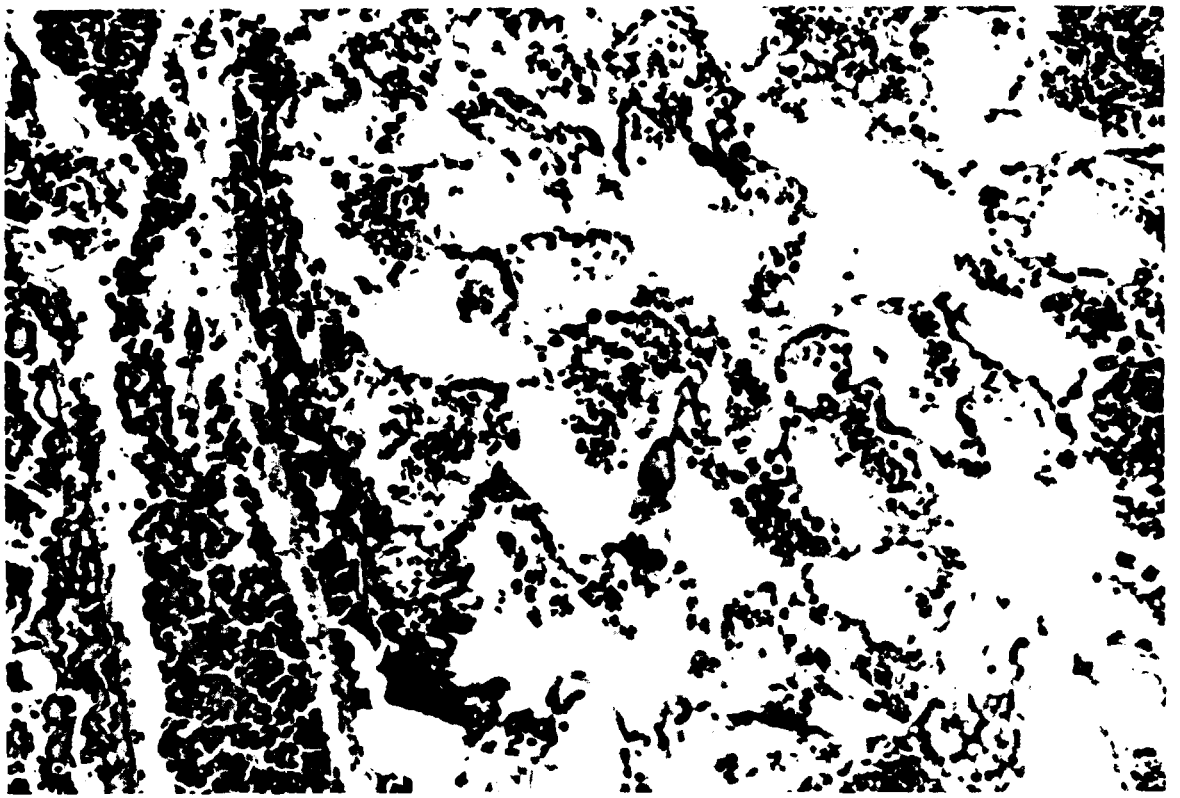

Figure 4b

Fig. 4. Pulmonary morphologic changes seen in canine lung after fresh (a) and old (b) blood perfusion; no significantly different changes were seen in morphology whether or not Dacron-wool filtration had been employed for the old blood (a: hematoxylin-eosin X 90; b: hematoxylin-eosin X 140).

Table 2. Morphologic changes noted with lung perfusion[1]

Blood	Interstitial edema	Perivascular hemorrhage	Alveolar hemorrhage	Intraalveolar fluid	Alveolar septum congestion
Baboon					
Fresh (24 hr)	2/6	0/6	1/6	0/6	2/6
Old (21 day)	7/7	7/7	7/7	7/7	7/7
Dacron-wool filtered old blood	4/7	3/7	4/7	2/7	6/7
Dog					
Fresh (24 hr)	2/7	2/7	1/7	1/7	3/7
Old (21 day)	7/7	6/7	6/7	5/7	6/7
Dacron-wool filtered old blood	6/7	4/7	4/7	3/7	5/7

[1]Number of lungs showing specified histologic finding/ number of lungs perfused

By at least several of the criteria, morphologic damage was judged to be present in most of the lungs perfused with stored blood, whether or not Dacron-wool filtered.

An interesting negative finding on examination of the histologic sections of lungs in the absence of demonstrable microemboli on histologic examination of lungs perfused with either fresh, stored or Dacron-wool-filtered stored blood. There was no consistent pattern that could be detected that indicated capillary obstruction or the mechanical impact of perfusion on the microvasculature of the lung was responsible for the functional changes seen in Table 1. Similarly, there was no perivascular cuffing of inflammatory cells or intravascular presence of aggregated blood debris.

DISCUSSION

In this animal model of in situ lung perfusion pulmonary morphologic and functional injury resembling that reported in the clinical syndrome of shock lung followed perfusion of these lungs with stored autologous blood. In the primate, elevation in the screen filtration pressure was noted with successively longer storage periods suggesting accumulation of microaggregated blood debris. From these facts alone, however, the conclusion that microaggregated blood debris is the single or even chief proximate cause of the pulmonary insufficiency that follows perfusion of lungs with autologous stored blood is not warranted. Several observations in these experiments with this lung perfusion model may indicate that microaggregates do not play a major role in the injury seen in pulmonary form and function.

There are marked differences in the microaggregation occurring during blood storage for different species, different storage techniques and preservatives, and different blood additives such as drugs, and differing pathologic events such as sepsis or factors released in shock. The latter factors were controlled in this experimental model, as the blood was proven sterile by culture, was not treated by any drugs except for the anticoagulants, and was perfused in an isolated perfusion circuit not representative of the circulation in shock.

There was a very marked difference between the observation of SFP during storage of blood from the primate and canine species. Figure 2 shows the climb in SFP determinations on stored baboon blood to immeasurably high values at 21 days, whereas no consistent elevation of SFP is noted in canine blood

stored under identical conditions in the same ACD preservative solution. The primate blood appears to mimic the SFP determinations seen with the storage of human blood in the same preservative for similar periods of time (5).

The preservative solution into which the blood is collected plays a major role in the degree of aggregation seen in the blood from each species. Microaggregates do form in canine blood when it is stored in heparin, so that SFP determinations on heparinized canine blood climb to immeasurably high values within the first 48 hr of storage. In a separate set of experiments with canine blood, the SFP determinations did not appear to rise in canine blood stored in ACD and, to a similar extent, citrate phosphate dextrose (CPD), but did occur rapidly in stored heparinized canine blood. Elevations of the SFP could be rapidly induced by the addition of adenosine diphosphate (ADP) to blood from either canine or primate species.

There may also be a qualitative difference in the aggregates measured by SFP or counted by particle counters that determine particle size and volume. There are soft aggregates, red cell rouleaux, platelet clumps or hard polymers of cellular debris that may form concretions later in storage as protein denaturation occurs along with continuing degeneration of cellular formed elements during blood storage. It is possible that aggregation might be seen in stored blood by the SFP determination, but that deaggregation occurs soon after infusion into the pulmonary perfusion circuit. This might particularly be true of the aggregates seen in primate blood when fresh cells are resuspended in plasma from stored blood, and this plasma might be somewhat diluted by the fresh blood in the priming of the perfusion circuit. However, SFP determinations were made on both the pulmonary artery and pulmonary vein sides of the lung, and no differences were seen to suggest that aggregation occurred on one side of the pulmonary capillary bed and deaggregation might take place on the other side of the same bed.

A causal correlation between high SFP in perfused blood and pulmonary functional and morphologic changes would not apply to the canine perfusion model and might have validity only when studying the baboon. However, the changes seen in the injury to lung form and function were the same in dog and baboon, without a corresponding similarity in SFP changes that could suggest microaggregates as a common factor.

The modifications in the perfusion protocol for the primate blood were designed to test the hypothesis that microaggregates might be responsible in at least the primate species for the pulmonary injury demonstrated after stored blood perfusion, and

modification in the stored primate blood that would reduce the microaggregates reflected in the SFP were attempted. Dacron-wool filtration did decrease the SFP in stored primate blood. Filtration of the stored primate blood did reduce the degree of lung damage according to some physiologic measurements, but Dacron-wool filtration did not render old blood innocuous to the lung.

Similarly, if the aggregated blood debris were carried in the cellular fraction of stored blood, separation of the old blood into packed cells and plasma fractions might allow isolation of the aggregates. When fresh blood cells were resuspended in the plasma from the stored blood, the SFP was seen to rise, but not to the immeasurably high level seen in the whole, old primate blood. Some pulmonary damage did result from perfusion with plasma from stored blood added to fresh cells. Some factor in the plasma from stored blood may possibly stimulate some microaggregation in the fresh cells to account for the injury. However, the noxious factor in the stored blood plasma might be implicated as a cause of the pulmonary injury directly, not necessarily mediated by its effect in stimulating microaggregation in the cellular fraction. In the primate perfusion model, pulmonary injury does result from the use of stored blood that can be mediated to some extent by the plasma fraction of old blood and cannot be eliminated by Dacron-wool filtration (6).

Regardless of microaggregation, microembolization could not be supported in either species on histologic examination of the lung, since no microemboli were seen, and no consistent pattern could be detected indicating capillary obstruction or a primary mechanical impact on the microvasculature of the lung as a primary cause of the pulmonary injury in both species. Even when the canine lung is infused with heparin-stored canine blood with an immeasurably high SFP, and changes in ventilation and vascular resistance follow, no microemboli can be confirmed by histologic examination of the canine lungs (7).

The canine lung perfusion data may be summarized by saying that damage to lung form and function was readily apparent following perfusion of stored canine blood which was not modified by depth filtration, and that these changes appeared to be independent of microaggregation/microembolization, since no elevation in SFP could be demonstrated in stored canine blood and no histologic evidence confirmed microembolization (8).

In primate lung perfusion, SFP elevations were noted in stored blood which could be reduced by Dacron-wool filtration, and to a lesser extent, by aged-cell separation. However,

filtration and aged-cell separation of stored blood did not completely abrogate the deleterious effect of perfusion on primate lung. A toxic factor apparently borne in the stored plasma appears to be implicated in both species as a possible factor in the cause of the pulmonary damage; this factor may operate in the primate at least in part by stimulating microaggregation, but it may also function independent of microaggregation in the primate as it seems to in the canine model.

SUMMARY

An in situ lung perfusion model was developed in canine and primate investigation of the impact of stored autologous blood on morphologic and functional derangement in the lung. Blood was drawn into ACD solution and stored either 24 hr or 21 days before culturing, standard recipient set filtering and perfusion through the left lower lung lobe equipped to monitor pressures and gas tensions in pulmonary artery, vein and bronchus. Observations of the primate and canine lungs were made during perfusion of fresh, old, Dacron-wool filtered old blood, and additionally, in the case of the primate, blood reconstituted by suspending fresh cells in plasma from stored blood.

Elevation in SFP was demonstrated only in the primate-stored blood, but stored blood in both species elevated the pulmonary vascular resistance, decreased the effective compliance and lowered the arteriovenous oxygen gradients, whether or not the stored blood was Dacron-wool filtered. Wet/dry lung weight ratios were increased and gross and microscopic evidence of pulmonary edema was seen in lungs perfused with stored blood; but no microemboli were seen by histologic examination.

The conclusion is reached that stored blood can cause injury to primate and canine lungs, but microaggregated blood debris is not the cause in the canine model and probably not the primary factor in the primate model. Some plasma-borne toxic factor might more probably be implicated as the common cause of the damage to lung form and function seen in each species following perfusion with autologous stored blood.

Acknowledgement

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MICROAGGREGATES AND PULMONARY INSUFFICIENCY

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Collins (12) has discussed the manifold nature of the causes of posttraumatic pulmonary insufficiency (PTPI). While a single insult of sufficient severity may induce this abnormality, modern therapeutic methods have minimized the occurrence of these singularly detrimental happenings.

A myriad of physiologic changes occur in the posttraumatic period either as a result of the traumatic insult or of therapy rendered. When each change is taken alone, the body may well accommodate to many such changes. However, when physiologic compensatory mechanisms have been compromised, it is possible that several of these sublethal events may all act synergistically. The pulmonary manifestation of such happenings may be PTPI.

Research in these laboratories has for some time attempted to define more specifically the interrelationship of only one of these multiple factors, microaggregates administered in blood transfusions, with the induction of PTPI.

Microaggregate Formation

It has been known since as early as 1948 (15, 59) that in blood stored under standard blood bank conditions, microscopic particles begin to appear within hours after collection and progressively accumulate during the storage period (41, 44-46, 52, 53, 57-59, 61). Such microaggregates form in human blood stored under standard blood bank conditions at 4°C in either acid citrate dextrose (ACD) or citrate phosphate dextrose (CPD) solution, and vary in size from 15 to 200 μ (1, 2, 20, 21, 40, 41, 44, 45, 51, 54, 57, 58).

During the first week of storage microaggregates formed consist mainly of platelets. Subsequently, granulocytes begin to disintegrate and degenerate, lose their cell membranes and adhere to the platelets (33, 38, 62). Fibrin then precipitates out of solution and adheres to these aggregates. Using a Coulter counter, the presence of up to 10^5 such aggregates per milliliter has been demonstrated in 14-day-old blood previously filtered through a standard blood transfusion filter with 170 μ pores (14). These microaggregates are compact in nature and are resistant to dissociation (36, 55).

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Improvements in the methods for collection, preservation and storage have failed to prevent accumulation of such microaggregates in stored blood. Indeed, microaggregate formation in stored packed cells is even more intense than in whole blood stored under comparable standard blood bank conditions (5).

Blood Transfusion and Microaggregates

Standard blood transfusion filters were developed for the removal of gross blood clots, and it has been known for some time that many microaggregates pass through these standard clot filters (15, 19, 34, 62).

In 1961 Swank discovered that an inordinately high pressure was needed to force stored blood through a 20 μ metal mesh screen (55). Since the normal constituents of blood are rarely greater than 15 μ in size, he concluded that the pressure was caused by the presence of microaggregates in the blood. Based on this principle he perfected the screen filtration pressure (SFP) method for determining the quantity of aggregates present in stored blood (55, 59).

In 1964, Swank and Porter (59) demonstrated that the SFP of blood circulated through cardiopulmonary bypass patients was substantially less in the venous line of the pump than in the arterial line. They concluded that this was due to filtration of microaggregates by the patient's microvasculature and asserted that such microembolism was harmful to these patients. This hypothesis was verified by Jenevein and Weiss (32) who isolated intact microaggregates from the myocardium and kidneys of patients who had undergone cardiopulmonary bypass procedures. A striking improvement in mortality in patients undergoing extracorporeal circulation was noted when introduction of a Dacron-wool filter permitted removal of these particles (3, 28, 58).

The absence of pulmonary insufficiency in patients receiving large amounts of stored blood from which microaggregates had been removed has also been emphasized (7, 8, 12, 13, 16, 32, 46, 48, 57). Blaisdell et al. (9) reported pulmonary microembolism to be a major cause of morbidity and death in patients undergoing reconstructive vascular surgery. Reul et al. (48) noted the absence of pulmonary insufficiency in a large group of such patients receiving blood transfusions when microaggregates were removed from the transfused blood using micropore filters. Hardy and Bane (25) have recently reported a patient who was given 116 units of blood within 4 days and showed no signs of pulmonary insufficiency, an extremely unusual occurrence. They attributed this absence of pulmonary complications to their having administered all blood through Dacron-wool transfusion filters.

In the Vietnam conflict, type-specific- or universal-donor-stored blood in unlimited quantities for treatment of casualties was available for the first time. Resuscitation and treatment of such patients

was associated with high incidence of respiratory insufficiency (35). In this patient population, Moseley and Doty found many who had pulmonary microemboli (45, 46). McNamara et al. (44) further demonstrated a correlation in such patients between the presence of microaggregates and the occurrence of arterial hypoxemia and concluded that this resulted from administration of banked blood. They further demonstrated microemboli plugging the pulmonary capillaries of those patients who died following injury and transfusion (42, 43).

Experimental Data

If pulmonary microembolism occurs during transfusion then one might expect that massive transfusion with blood containing microaggregates would be associated with pulmonary hypertension (7, 8, 29, 30, 48). In early experiments in these laboratories, animals underwent exchange transfusions of approximately twice blood volume using microaggregate rich blood (16). The SFP measurements verified the presence of large numbers of aggregates in the transfusions. The blood was given using no filter, standard blood transfusion filters or Dacron-wool blood transfusion filters. When either no transfusion filters or standard blood transfusion filters (pore size 170 μ) were used, experimental animals developed pulmonary hypertension, and total body O_2 consumption decreased. These detrimental changes did not occur when microembolization was prevented using Dacron-wool filters.

In more closely controlled experiments in which animals underwent exchange transfusions equal to blood volume, it was demonstrated that standard filters reduced SFP of the stored blood somewhat (4). However, numerous microaggregates passed the filters, and post-filtration SFP of the blood remained high. After transfusion, average O_2 consumption decreased. Pulmonary arterial hypertension was associated with an increased pulmonary arteriovenous shunting (Q_s/Q_t) of blood and a decrease in pulmonary diffusion capacity (DO_2). The presence of extensive numbers of microemboli in the pulmonary arteriolar and capillary bed was confirmed by microscopic examination of lung tissue. Animals were followed for several hours after completion of the exchange transfusion, but all experiments were fatal.

On the basis of this research it appeared reasonable to assume that transfusion of sublethal quantities of microaggregate-rich blood might lead to the development of progressive changes. If the animal's compensatory mechanisms were adequate, then resolution of these changes might occur and pulmonary function might return to normal.

Ten dogs underwent partial exchange transfusions ranging between 35% and 78% of blood volumes (average 60%) through standard blood

transfusion filters (11). Average SFP of the blood after filtration was elevated to 85 mm Hg. Pulmonary hypertension did not develop, but there were striking decreases in O_2 consumption, increases in Q_s/Q_t and decreases in DO_2. The Q_s/Q_t increase correlated directly with the quantity of microaggregates given (Fig. 1). Changes became progressively more marked over the first 48 - 72 hr after the transfusion and then progressively returned nearly to normal by day 6 after transfusion. Pathologic examination of the lungs of animals sequentially sacrificed over 6 days showed intravascular microemboli, alveolar cell hyperplasia and interstitial and alveolar pulmonary edema. Progressive recovery of pulmonary function was associated with progressive resolution of detrimental pathologic changes (Fig. 2). It was worthy of note that in six dogs exchange-transfused 100% of their blood volume through Dacron-wool filters and in three control animals that were not transfused, there were no significant changes in pulmonary structure or function.

In other research, patients who received intraoperative blood transfusions through standard blood transfusion filters were studied and compared with patients who received comparable quantities of blood through Dacron-wool filters or who received no blood transfusions (6). The changes observed in Q_s/Q_t in each patient were correlated with a quantity of microaggregates administered to that patient (Fig. 3). A direct correlation was found between this quantitative value derived for each patient and the observed change in Q_s/Q_t in that respective patient (Fig. 1).

Mechanisms of injury

The possible role of microembolism in producing pulmonary insufficiency has been explained by Blaisdell et al. (9, 10) and Brown et al. (11). Quite possibly these changes are due to a progessive unremitting deterioration through a chain of events characterizing a "vicious cycle" (Fig. 4). Occlusion of the pulmonary microvasculature and direct damage to the pulmonary capillary endothelium probably constitute the initiating mechanisms (56).

Occlusion of the pulmonary microvasculature is due in large part to mechanical blockage by the microaggregates. Proximal arteriovenous shunts open, and deoxygenated blood enters the pulmonary veins and the systemic ciruclation producing hypoxia. When the administered microaggregate load is sufficiently great, pulmonary hypertension develops. As pulmonary shunting increases, hypoxia becomes progressively more severe. Opening of the arteriovenous shunts also leads to an increase in the pressure at the venous end of the pulmonary capillaries and interstitial pulmonary edema develops (Starling's Law of Capillary Pressure). This interstitial edema enters the alveoli and alveolar pulmonary edema further inhibits oxygenation.

Other factors may also be of importance. Serotonin, a powerful vasoconstrictor, is present in high concentrations in platelets and

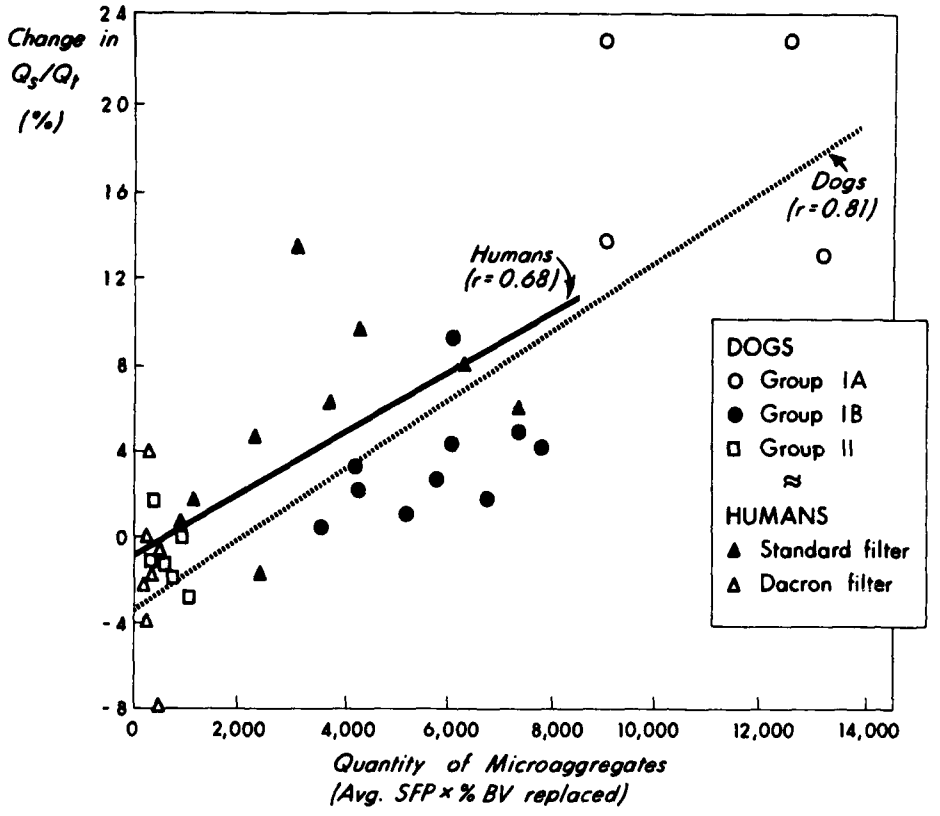

Figure 1. Relationship between changes in pulmonary arteriovenous shunting (Q_s/Q_t) and degree of pulmonary microembolization during blood transfusion. There was a direct correlation between the absolute per cent change in Q_s/Q_t and the quantity of microaggregates administered. All animals transfused up to 100% of blood volume through standard transfusion filters (Group 1A) died from pulmonary insufficiency. Graded responses of lesser severity were produced in other animals by giving smaller quantities of microaggregates (Group 1B). Animals transfused through dacron wool (Swank) micropore filters showed statistically insignificant changes in Q_s/Q_t (Group II).

The same pattern was observed in humans transfused through either standard or dacron wool (Swank) filters.

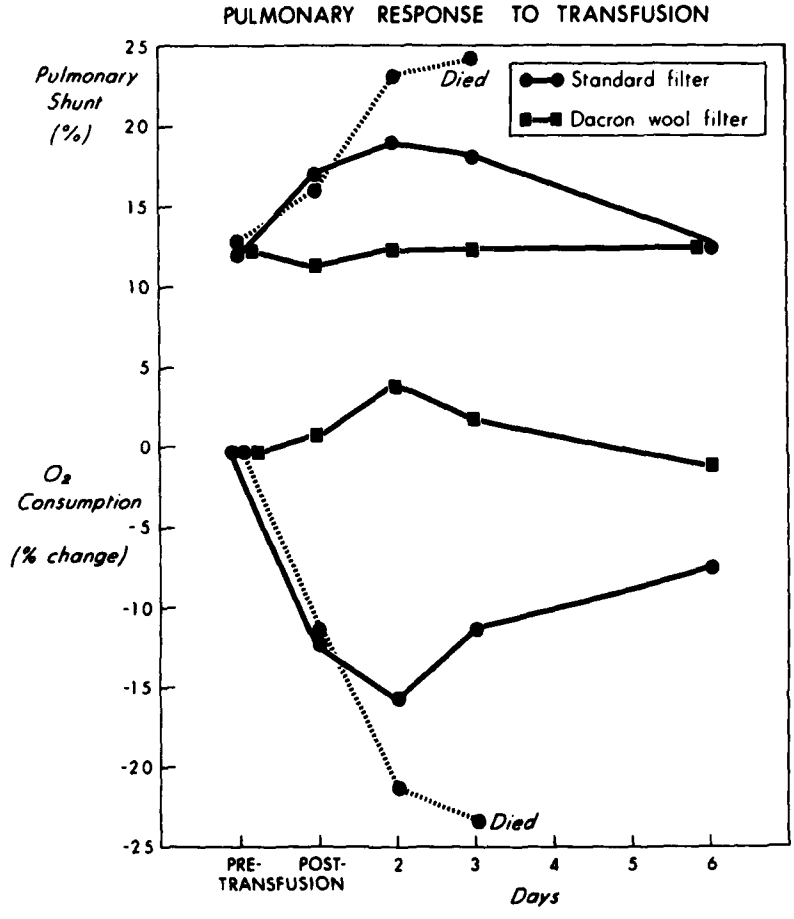

Figure 2. Pulmonary response to blood transfusion. When a limited quantity of blood with an elevated screen filtration pressure was infused to dogs through standard blood transfusion filters, a marked increase occurred in pulmonary arteriovenous shunting. This led to a decrease in total body O_2 consumption. Changes appeared to be most severe 48 to 72 hours after the transfusions, and one animal died on the fifth day after transfusion before pulmonary function tests could be repeated. In the survivors pulmonary function had returned almost to normal by the sixth post-transfusion day. Detrimental changes did not occur when dacron wool (Swank) blood transfusion filters were used. (From Brown, C., et al: Progression and Resolution of Changes in Pulmonary Function and Structure Due to Pulmonary Microembolism and Blood Transfusion. Ann. Surg., 185:92, 1977).

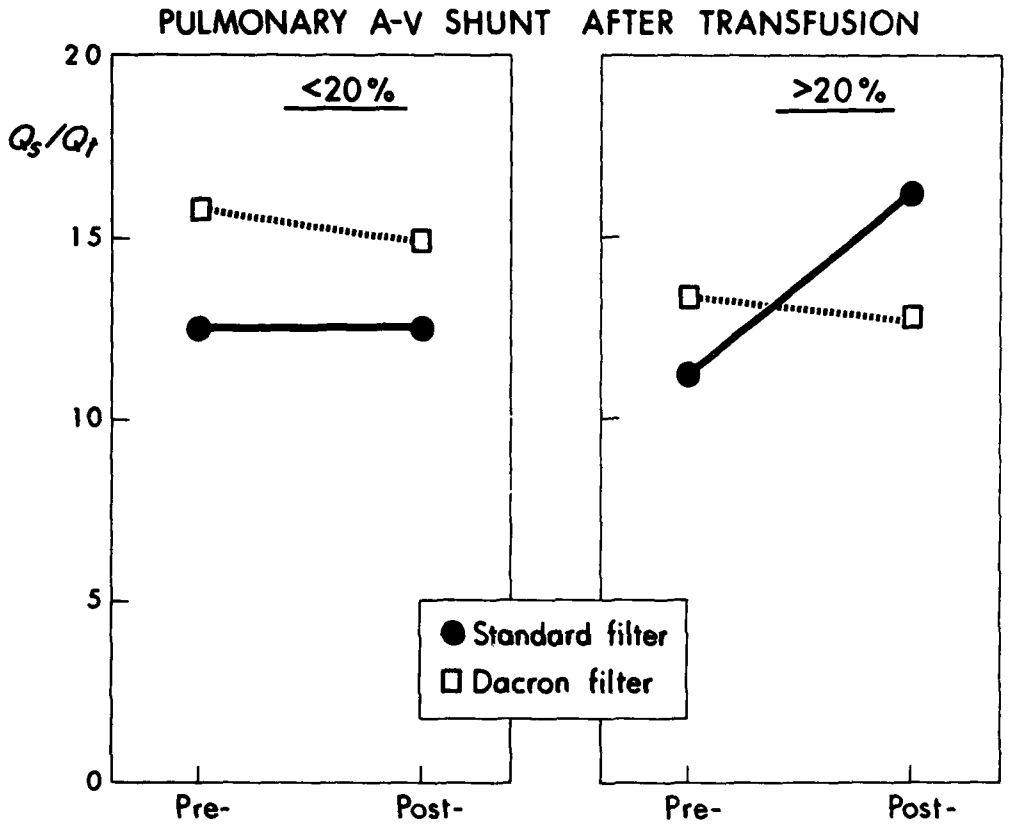

Figure 3. Pulmonary arteriovenous shunt in patients after transfusion. Transfusions of less than 20% calculated blood volumes through either standard (3 patients) or dacron wool (Swank) filters (3 patients) or over 20% of calculated blood volumes through dacron wool (Swank) filters (6 patients) did not lead to increase in average Q_s/Q_t.

Average Q_s/Q_t increased significantly ($p < 0.01$) when transfusions of over 20% of calculated blood volumes were given (eight patients, average SFP=85 mm Hg).

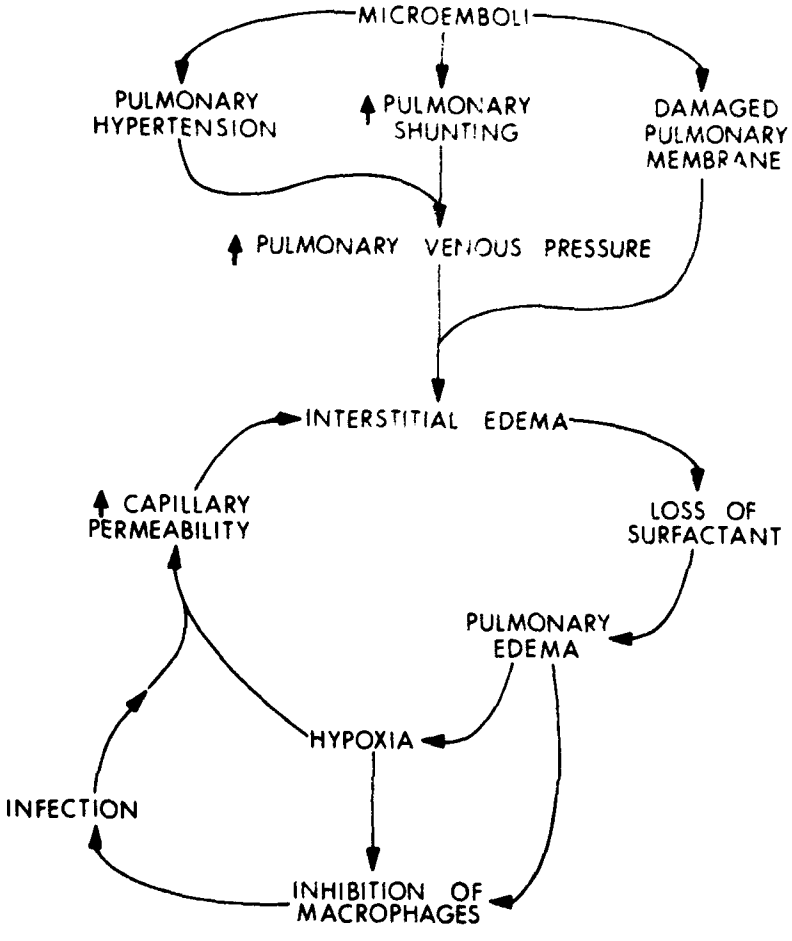

Figure 4. "Vicious Cycle" that may be initiated by transfusion containing microaggregates. (From Brown, C., et al: Progression and Resolution of Changes in Pulmonary Function and Structure Due to Pulmonary Microembolism and Blood Transfusion. Ann. Surg., 185:92, 1977).

may be released by disintegrating platelets directly into the pulmonary microvasculature.

Microaggregates may also damage the pulmonary membrane directly by release of lysosomal enzymes, the active ingredient of intracellular lysosomes. Disintegrating leukocytes and platelets in the microaggregates or, alternatively, damaged cells in the pulmonary membrane, may be the source of this lytic material (13, 17, 31). The damaged alveolar membrane then allows fluids, proteins and red cells to leak into the interstitial spaces and alveoli and to inhibit oxygenation still further.

Increased pulmonary shunting and transudation from pulmonary capillaries lead to decreased perfusion of alveolar lining cells, anoxia of the granular pneumonocytes, and subsequently, impaired phospholipid synthesis and surfactant production (24, 26, 27). Furthermore, pulmonary transudates and exudates such as pulmonary edema, blood and plasma inactivate surfactant (49, 50, 60). Inactivation of surfactant and impairment of its synthesis result in an unstable alveolus and lead to alveolar collapse, atelectasis and further transudation. The result is further inequalities in ventilation:perfusion, a further increase in the right to left pulmonary shunt and even more severe hypoxia.

Atelectatic lung tissue is more prone to develop infection than is the tissue of normal lung. When such occurs, further damage to the pulmonary membrane, further transudation, etc., may result. Since collapsed alveoli mechanically interfere with capillary blood flow, atelectasis may also increase pulmonary vascular resistance (47).

Alveolar macrophages have a high level of aerobic glycolysis, and particle uptake is inhibited by low oxygen tension (23). Thus, in the presence of hypoxia, pulmonary infection becomes more likely. When such occurs further pulmonary damage, atelectasis, and ultimately, more severe hypoxia are produced.

By causing pulmonary vasoconstriction, an increase in capillary permeability (18) and further intraalveolar transudation, hypoxia enhances many of the pathologic processes in progress. Gradually hypoxia resulting from this "vicious cycle" becomes increasingly more severe until it becomes refractory to treatment.

Discussion

During storage blood for transfusion progressively accumulates increasing numbers of microaggregates (41). Many of these microaggregates are not removed by standard transfusion filters which were developed for the removal of gross blood clots. In spite of considerable experimental as well as circumstantial evidence, disagreement continues to exist as to whether or not such particles

are related to the development of PTPI (22, 37, 39). Perhaps the reason for this uncertainty has been the failure to recognize that microaggregates may be a contributory cause as well as a primary cause. In addition, many other factors such as pulmonary contusion, fat embolism, atelectasis, etc., may also produce or contribute to development of the same syndrome.

The pulmonary microcirculation can accommodate a large microaggregate load (22), and pulmonary changes in animals due to microaggregates are clearly dose-related (Fig. 1). Such changes can be completely prevented by removing microaggregates from transfusions. On the basis of these data a small patient with a relatively small pulmonary vascular bed would manifest pulmonary changes of greater severity than a larger patient with a large pulmonary vascular bed receiving the same amount of blood containing the same quantity of microaggregates. A patient receiving blood relatively free of microaggregates would demonstrate fewer physiologic changes than an individual with the same size pulmonary vascular bed receiving the same amount of blood transfusion but containing greater numbers of microaggregates. The individual with severely restricted pulmonary compensatory mechanisms would demonstrate a more exaggerated response to the same quantity of microaggregates than would a healthy patient without such compromised pulmonary function.

Pulmonary changes caused by transfusion of graded quantities of microaggregate-rich blood may lead to pulmonary complications that are not necessarily fatal. Studies in these laboratories have demonstrated complete recovery without pulmonary hypertension in animals transfused up to 75% of their blood volume using blood with an average SFP of 85 mm Hg. If the abilities of the lung to respond to physiologic stress have not been previously compromised, external signs of respiratory abnormalities may be so subtle that sophisticated physiologic measurements may be necessary to demonstrate any changes.

Extensive in vitro testing has demonstrated removal of large numbers of microaggregates from banked blood by micropore filters. Whether or not such microaggregates should be removed from a blood transfusion seems to this author to be a rhetorical argument. Were this material present in an intravenous solution rather than a blood transfusion, few physicians would consider it advisable to administer such a solution. Furthermore, no one has ever demonstrated that microaggregates in stored blood are beneficial or that transfusion of microaggregate-rich blood leads to an improvement in pulmonary function.

Experiments in animals have demonstrated the relationship between infusion of microaggregates and the development of pulmonary insufficiency. To demonstrate the production of this abnormality in humans would be not only difficult but also unethical. To administer such material to patients in an infusion of any sort constitutes an unnecessary and unjustifiable danger.

Several types of relatively inexpensive micropore filters are now commercially available. Even though one type may function more efficiently and effectively than the others, all remove large quantities of microaggregates, and none cause more damage to the formed elements of the blood than do standard transfusion filters. It is the opinion of this author that before blood is administered to patients, it must first be determined that microaggregates are not present or the blood must be administered through an effective micropore transfusion filter. Adequate data exist to emphasize this important aspect of modern blood transfusion.

Conclusions

Through mechanisms that have not yet been clarified, microaggregates administered during blood transfusion may lead to the development of pulmonary insufficiency. The severity of the changes induced are dose-related. When small doses of microaggregates are administered, changes may be so subtle that they may escape detection. Pulmonary changes are most apt to occur in patients in whom normal physiologic compensatory mechanisms have already been compromised either by preexistent disease or several sublethal insults associated with multiple trauma or its treatment.

In order to minimize this hazard, it is the author's opinion that before a blood transfusion is administered, it must first be determined that microaggregates are not present or the blood must be transfused through an effective micropore blood transfusion filter.

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THE EFFECT OF MICROAGGREGATES IN STORED BLOOD ON THE CANINE PULMONARY VASCULAR BED

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INTRODUCTION

The etiology of the adult respiratory distress syndrome (RDS) remains undetermined. Experimental studies have suggested a number of causative factors (2, 3, 9, 11). However, no consensus has developed implicating one factor as the agent that could initiate the pathologic changes characteristic of RDS. Clinical studies have been complicated by the multiplicity of factors involved in patients who develop RDS. Shock, sepsis, head trauma, long bone injuries and use of large amounts of intravenous fluid and blood transfusions in the resuscitative efforts frequently are present in a single patient with RDS. In these circumstances, it is difficult to determine the relative importance of the many factors involved.

Pulmonary trapping of microaggregates present in banked blood has been suggested as an important etiologic agent in the development of RDS (1, 5, 6, 8, 10). Presumably microaggregates act as micro-emboli obstructing the pulmonary capillary bed or release vasoactive hormones that affect the resistance of the pulmonary capillaries. Either of these mechanisms may set up secondary changes that develop into RDS.

This study evaluates the ability of microaggregates in stored blood to obstruct the pulmonary capillary bed. Stored autologous blood was transfused through a catheter placed in the left main pulmonary artery. Pulmonary blood flow distribution was determined before and after transfusion by the radioactive microsphere technique. A redistribution of blood flow away from the left lung into the right lung would indicate significant obstruction of the pulmonary capillary bed.

METHODS AND MATERIALS

Part I

Eight mongrel dogs, weighing approximately 25 kg each, were bled daily for 3 days and the blood was stored at 4°C in heparinized blood bags (Fenwal Laboratories, Deerfield, Ill.) for 3-6 days. The animals were anesthetized (pentobarbital, 30 mg/kg, iv) intubated, and given

intravenous heparin (250 U/kg). A Swan Ganz catheter with a Thermister probe (Edwards Laboratory, Santa Ana, Calif.) was placed through the external jugular vein and positioned in the pulmonary artery. Both femoral arteries were cannulated. The left femoral arterial catheter was connected to a pressure transducer (Statham Ray Instruments, Waltham, Mass.) and the right catheter was used for bleeding. Cardiac output was determined by the thermal dilution technique (Thermal Dilution Cardiac Output Meter, Columbus Instruments, Columbus, Ohio). Pulmonary blood flow distribution was determined by the injection of radioactively tagged microspheres (Tracer Microspheres Nuclear Products, 3M Company, St. Paul, Minn.) into the right atrium. Regional pulmonary blood flow was calculated by multiplying percent distribution by cardiac output.

Microaggregates were quantitated with the use of an electronic multichannel particle size analyzer (Coulter Counter Electronics Model T, Hialeah, Fla). This instrument determines the number and volume of microaggregates in 15 different particle diameters from 3 to 160 μ. Data concerning particles with diameter of 10 μ or less were excluded because of the presence of physiologic particles in that range. On the day of the experiment, samples were obtained from each unit of blood, passed through a standard 170 μ filter (Fenwal Laboratories, Deerfield, Ill.), and tested for microaggregates. For comparison, 16 units of 14-day-old human blood also were tested.

Following a 30-min stabilization period, the pulmonary arterial pressure, wedge pressure, and cardiac output were determined. The Swan Ganz catheter was pulled back to the right atrium and ^{141}Ce-tagged microspheres were injected for control pulmonary blood flow distribution (Period I). The Swan Ganz catheter was removed and, with use of fluoroscopy and angiography, another catheter was passed via the external jugular vein into the left pulmonary artery.

The animals were bled to an aortic pressure of 40 mm Hg and maintained at that pressure for 2 hr. They were resuscitated by the transfusion of the stored blood through the catheter in the left pulmonary artery. After resuscitation, the position of the catheter was again verified by x-ray and removed. The pulmonary arterial and wedge pressures and cardiac output were determined. Chromium 51-tagged microspheres were injected into the right atrium (Period II). Three hours following resuscitation, the measurements were repeated and ^{85}Sr-tagged microspheres were injected into the right atrium (Period III).

Both lungs and the right kidney were removed, sectioned, and the radioactivity in the tissue sections counted (Autogamma Spectrometer, Hewlett Packard, Palo Alto, Calif.). The absence of radioactivity in the right kidney verified that all the microspheres were trapped in the lungs. The (percent) distribution of cardiac

output was determined by dividing the counts in each lung by the total lung count. This was converted to flow by multiplying the percent distribution by the cardiac output. Pulmonary vascular resistance (PVR) in resistance units (RU) was calculated for each lung by the formula:

$$\frac{\text{Pulmonary arterial-wedge pressure (mm Hg)}}{\text{flow (cc/sec)}}$$

The data were treated as a multivariate problem of repeated measurements. The Hotellings T-square test was used to determine statistical significance.

Part II

Sixteen adult mongrel dogs weighing approximately 25 kg, were used in this section. The animals were bled daily for 3 days and blood was stored at 4°C in heparinized blood bags (Fenwal Laboratories, Deerfield, Ill.) for 3-6 days.

On the day of the experiment, the animals were prepared as in Part I. The first microsphere (^{141}Ce) was injected in the right atrium for control distribution of pulmonary blood flow. A catheter was placed in the left main pulmonary artery under fluoroscopy. The animals were then divided into two groups. In Group A, the autologous blood was passed through a standard 170 μ microfilter and transfused into the left lung. One hundred milliliters of blood were removed at appropriate intervals from the femoral arterial catheter to balance the infusion of stored blood. Pulmonary blood flow distribution and pulmonary vascular resistance were determined 0.5 hr and 3 hr following completion of the transfusion. In Group B (n=7), the animals were treated as in Group A except the blood was passed through a Swank microfilter before being transfused into the left lung. The pulmonary blood flow distribution and the pulmonary vascular resistance were compared between Group A (filtered with standard 170 μ filter) and Group B (filtered with Swank microfilter) to determine the effect of microaggregates on pulmonary hemodynamics.

In this section, microaggregates were also quantitated with the Coulter counter. Comparisons were made between human blood, canine blood filtered with 170 μ filter and canine blood filtered with a Swank microfilter.

RESULTS

Part I

The total volume of microaggregates up to 160 μ is 2,924 ± 5,425 u^3/mm^3 for canine blood and 8,223 ± 565 u^3/mm^3 for human blood. The volume and number of microaggregates in the canine blood were significantly greater than in the human blood ($p < 0.05$).

In the control period (I), the right lung received 57.6 ± 0.9% and the left lung 42.4 ± 0.9% of the cardiac output. One-half hour following resuscitation (Period II), the distribution essentially was unchanged with the right side receiving 57.8 ± 2.0% and the left 42.2 ± 2.0%. Three hours following resuscitation (Period III), 55.8 ± 1.3% went to the right and 44.2 ± 1.3% to the left. Statistical analysis showed that the distribution of the blood in Periods II and III was not significantly different from the control distribution in Period I ($p>0.05$).

The vascular resistance across the right lung was 0.180 ± 0.03 RU and the left 0.242 ± 0.040 RU (Period I). This increased to 0.431 ± 0.08 RU for the right and 0.594 ± 0.12 RU for the left (Period II) and 0.544 ± 0.08 RU for the right and 0.688 ± 0.11 for the left (Period III). The resistance across each lung in Periods II and III was significantly greater than that of the control ($p<0.05$). The percentage increase in resistance for the right lung was 139% in Period II and 202% in Period III, and for the left lung was 145% during Period II and 184% during Period III. The percentage increase across the right lung was not statistically different from the increase across the left lung ($p>0.05$). These data are summarized in Tables 1 and 2. During shock, a total of 974 ± 56 ml or 42% of the calculated blood volume was removed from the animal, and for resuscitation, 1,160 ± 47 ml of the stored blood was returned.

Table 1. Percentage distribution of cardiac output expressed as mean ± SEM, (part I).

	Period I	Period II	Period III
Right lung	57.6 ± 0.9	57.8 ± 2.0	55.8 ± 1.3
Left lung	42.4 ± 0.9	42.2 ± 2.0	44.2 ± 1.3

Table 2. Pulmonary vascular resistance expressed in resistance units as mean ± SEM (part I).

	Period I	Period II	Period III
Right lung	0.180 ± 0.03	0.431 ± 0.08	0.544 ± 0.08
Left lung	0.242 ± 0.04	0.594 ± 0.12	0.688 ± 0.11

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Part II

After filtration with a 170 μ filter, the total volume of microaggregates transfused to the dogs in Group A was 1871 ± 170 x 10^3 μ^3/cc of canine blood. The total number of microaggregates was 35,079 ± 6226 particles/cc of blood. In Group B, following filtration with the Swank microfilter, the total volume of microaggregates administered to the animals was 270 ± 100 x 10^3 μ^3/cc of blood and the total population was 8,603 ± 3383 particles/cc of canine blood.

AD-A092 838 ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND FORT D--ETC F/G 6/5
MICROAGGREGATES: EXPERIMENTAL AND CLINICAL ASPECTS - SYMPOSIUM --ETC(U)
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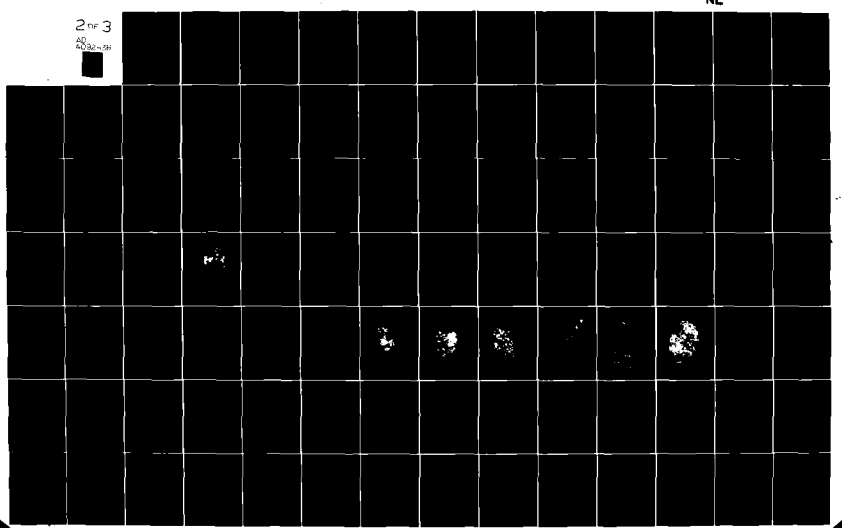

The volume and number of microaggregates in Group A are significantly greater than the microaggregates administered to Group B ($p < 0.05$).

In animals in Group A, the left lung received 43.96 ± 0.68% of pulmonary blood flow. Thirty minutes after receiving the microaggregates, the percentage was 42.59 ± 1.33 and 3 hr after the transfusion, the percentage of blood flow was 41.05 ± 1. In animals in Group B, control pulmonary blood flow was 42.89 ± 1.7%. Thirty minutes after transfusion it was 42.60 ± 1.6% and 3 hr following administration of the blood it was 43.93 ± 2.8%.

The resistance across the left lung was .204 ± 0.01 for Group A and .203 ± 0.01 for Group B during the control period. During period II, the resistance in animals in Group A increased to .314 ± 0.05 and in animals in Group B to .447 ± 0.07. During period III, the vascular resistance in animals in Group A was .480 ± 0.14 and in Group B .489 ± 0.07.

The change in the percentage of pulmonary blood flow to the left lung in Group A from period I to period III was not statistically different from that recorded for the animals in Group B. The vascular resistance change for the left lung in Group A was not statistically different from that in Group B. These data are summarized in Tables 3 and 4.

In Group A, the animals received 1298 ± 30 ml of stored blood and in Group B, the animals received 1305 ± 42 ml of stored blood.

Table 3. Percentage of cardiac output to left lung expressed as mean ± SEM (part II)

Group	Period I	Period II	Period III
A	43.96 ± 0.68	42.59 ± 1.33	41.05 ± 1.0
B	42.89 ± 1.7	42.60 ± 1.60	43.93 ± 2.8

Table 4. Pulmonary vascular resistance of left lung expressed as mean ± SEM (part II)

Group	Period I	Period II	Period III
A	.204 ± 0.01	.314 ± 0.05	.480 ± 0.14
B	.203 ± 0.01	.447 ± 0.07	.489 ± 0.07

DISCUSSION

Microaggregates are clumps of platelets and leukocytes that form in human blood following storage in acid citrate dextrose (ACD) or citrate phosphate dextrose (CPD) solutions (7). These particles range in size from less than 3 μ to visible macroparticles. Particles less than 10 μ are equal to the size of leukocytes and are too small to obstruct the pulmonary capillary bed. Particles greater than 170 μ are removed by the standard filter found in blood administration sets. The particles ranging in size from 10 to 170 μ have been implicated as important in the development of RDS. Canine blood does form appreciable quantities of microaggregates if stored in a heparinized bag.

The amount of microaggregates per millilter of stored canine blood was a least three times that found for each milliliter of stored human blood. In clinical practice we transfuse blood through a peripheral vein and microaggregates are distributed to both lungs in proportion to the percentage of cardiac output that lung receives. In our preparation, the microaggregates were transfused only to the left lung which received 42% of the cardiac output. In clinical practice for the left lung to receive the amount of microaggregates we gave, more than twice that amount would have to be administered through a peripheral vein. The large amounts of microaggregates in canine blood, the transfusion directly to the left lung and amount of blood transfused compared with the blood volume of the animals suggest that in our preparation we administered enormous quantities of microaggregates.

This study suggests that microaggregates in large quantities are not sufficient to mechanically obstruct the canine pulmonary bed. In the hemorrhagic shock group, no redistribution of blood flow from the left lung to the right lung occurred following the transfusion of microaggregates. This indicates the pulmonary vascular resistance of the left lung relative to the right lung did not change. It is possible that the systemic acidosis secondary to hemorrhagic shock could have masked subtle effects of microaggregates on the pulmonary circulation. To rule this out, we repeated the experiment without placing the animals in hemorrhagic shock. In the A group, the blood was transfused through a 170 μ filter. In the B group, the blood was transfused through a Swank microfilter essentially removing all the microaggregates. Only a slight decrease in the percentage of blood flow to the left lung in Group A following transfusion occurred. However, the pattern of pulmonary blood flow was not statistically different than that observed in Group B.

Although the number of microaggregates in human and canine blood is impressive, it is small in comparison to the enormous size of the pulmonary capillary bed. It is estimated that in the dog there are 600,000,000 capillaries with a cross-sectional area of 23,100 mm^2 (4).

A similar capacity in man enables him to increase cardiac output from 4 to 30 L/min with no significant change in pulmonary arterial pressure. These observations make it unlikely that microaggregates are present in sufficient quantities to alter pulmonary hemodynamics by mechanically obstructing the pulmonary capillary bed.

SUMMARY

Blood flow and resistance changes are compared between the right and left lung following the selective administration of stored autologous heparinized blood into the left pulmonary artery. Two different experiments were done. In part I, the stored autologous blood was administered following a 2-hr hemorrhagic shock period. In part II, an exchange transfusion was done with one group of animal receiving blood filtered with 170 μ filter and the second group receiving blood filtered with a Swank microfilter.

Despite the administration of massive amounts of microaggregates into the left pulmonary artery, the distribution of blood flow to the left lung did not change significantly. These data suggest that microaggregates do not mechanically obstruct the pulmonary capillary bed.

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THE FLOW PROPERTIES OF BANKED BLOOD

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INTRODUCTION

Respiratory failure has emerged as one of the most common causes of death following major trauma or surgery (1-5). A long list of names has been assigned to this syndrome in an attempt to define and describe it. Among these are traumatic wet lung (7), hemorrhagic atelectasis (4), shock lung (6), postperfusion pulmonary congestion (2), posttraumatic pulmonary insufficiency (20), respiratory distress syndrome of shock and trauma (6), and others. In spite of the lack of a clear understanding of the basic pathophysiologic mechanism(s) involved in these syndromes, the association between pulmonary insufficiency, multisystem organ dysfunction and the administration of large volumes of banked homologous blood is well recognized. The aggregates of amorphous material which develop during the storage of human blood have been implicated as a source of extensive pulmonary microembolism following massive transfusion and this has been cited as an important factor contributing to the development of the ensuing respiratory difficulties (19,21). Clinical and laboratory studies support this concept, and have identified the amorphous particulate material to be primarily platelets, leukocytes, and fibrin.

During the course of rheologic studies using buffy-coat-poor banked blood, significant abnormalities in flow properties were discovered after a period of time far shorter than the accepted shelf life, and a systematic evaluation of these properties was therefore undertaken.

MATERIALS AND METHODS

The blood for these studies was obtained from healthy adult human volunteers using standard blood bank techniques, including constant agitation of the blood during collection. A total of 15 units of

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whole blood was used. All were collected in commercial plastic bags containing NIH formula A preservative-anticoagulant, more commonly known as ACD acid citrate dextrose (ACD, Cutter Laboratories, Berkeley, California). The blood was stored at 4°C for 21 days, during which, aliquots were removed at intervals. Each bag was thoroughly mixed prior to removal of samples. The following rheologic variables were quantified:

1. Whole blood viscosity over a shear rate range of 0.1 to 230 inverse seconds (sec^{-1}), using Couette (G.D.M. and Contraves Low-Shear) and coneplate (Wells-Brookfield) viscometers. Viscosity values are given in centipoise (cp).
2. Plasma viscosity (cp) at high shear rates (230 inverse seconds). Plasma was prepared by centrifugation of whole blood at 10,000 X g for 10 min which effectively removes all cells.
3. Erythrocyte fluidity (the reciprocal of viscosity) of concentrated red cell suspensions with hematocrits between 95 and 99%, prepared by multiple centrifugations until the desired hematocrit was achieved. Due to the high viscosity of these samples, their viscosity was measured only on the Wells-Brookfield viscometer at 230 inverse seconds.
4. Filterability of dilute (2% hematocrit) red cell suspensions through polycarbonate sieves (Millipore type SM filters, Millipore Corp., Bedford, Massachusetts) having a mean entrance pore diameter of 5.0 $\pm$ 0.3 μm. The erythrocyte-plasma suspension was driven through the filters under standardized conditions (37°C, 15.0 $\pm$ 1.0 cm H_2O driving pressure) and the flow time for 2 ml of this suspension measured by a stop watch. This provides an excellent measure of erythrocyte deformability.
5. Morphology of the erythrocytes using phase contrast microscopy. Red cells were counted and classified as biconcave discs (normal), crenated discs, crenated spheres, or spheres (spherocytes) and the percentage of each calculated.

All of the determinations were performed at 37°C except for the morphologic ones which were done at room temperature.

Nine of the 20 units of blood were studied rheologically both before and after the passage of each aliquot through a Dacron-wool filter (Swank 1L 200, Pioneer Filters, Beaverton, Oregon), using gravity flow.

RESULTS

Due to considerable variability between individual units of blood, all results will be given as percentage change $\pm$ SEM from control values. Statistical comparisons were made using Student's *t*-test.

Whole blood viscosity at a high shear rate of 230 inverse seconds was increased by 16.7 ± 4.1% after 5-7 days storage; by 23.8 ± 3.9% after 14 days storage; and by 30.5 ± 3.5% after 21 days storage in ACD (Fig. 1). These values are all statistically significantly different from controls (day of blood donation), $p<.05$. The percentage increase after Dacron-wool filtration was 9.5 ± 2.0%, 14.0 ± 2.6%, and 22.8 ± 3.0% after the same respective time periods. These values are also all statistically different from controls ($p<.05$) but not from the unfiltered samples.

Whole blood viscosity near stasis, shear rate 0.1 inverse seconds, showed only very slight changes with storage (Fig. 2). The 10.0 ± 2.5% decrease in viscosity observed after filtration of 21-day-old blood, although slight and not statistically significant, was present in every sample.

Plasma viscosity measured at 230 inverse seconds did not change significantly although it was increased by 7.0 ± 1.0% by the end of 21 days.

Red cell fluidity as measured by the viscosity of packed red cell suspensions was increased by 59.4 ± 6.1% after 5-7 days; by 94.1 ± 9.0% after 12-14 days; and by 65.0 ± 9.0% after 21-days storage. These values differ significantly from control values, ($p<.05$), but were unchanged by Dacron-wool filtration.

Erythrocyte deformability showed the most striking changes with storage time. Within 5 days of storage, the rate of passage of the red cell suspensions through the 5 μm filters was reduced by 46.0 ± 5.1% (Fig. 3). By 14 days there was a 91 ± 10.1% reduction in flow and from about day 18 on, there was essentially no passage of cells through the filters. These values are statistically significantly different from controls ($p<.05$) but were unaltered by Dacron-wool filtration.

Striking changes in erythrocyte morphology were also apparent by day 7 of storage, when only 46.0% of the cells retained their biconcave disc shape (Fig. 4). There were essentially no normal appearing red cells by 21 days. There was a progressive increase in the proportion of crenated spheres to 30.0% at 14 days and 48.0% at 21 days. The same was true for crenated spheres but to a lesser degree. Spheres did not appear until after day 7 of storage and then progressively increased to 37.0% by the end of 21 days. Dacron-wool filtration did not alter these relative percentages.

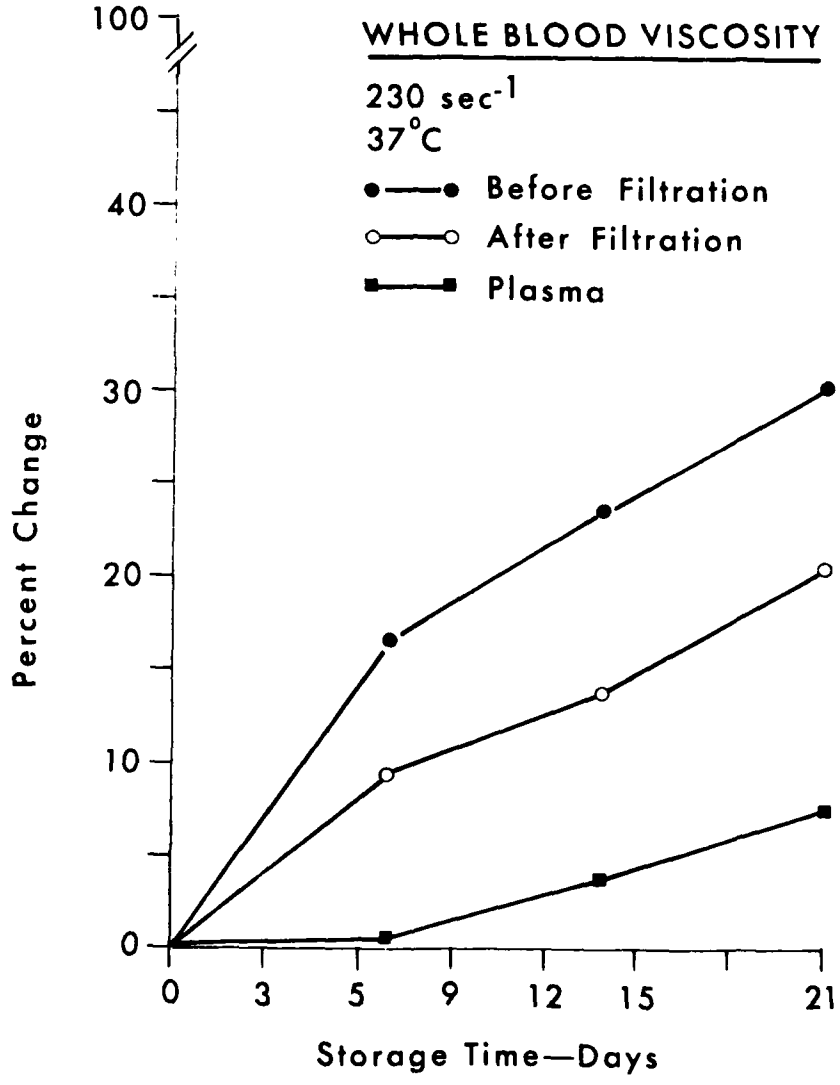

Fig. 1. Percent change during 21 days storage at 4°C of whole blood, and plasma viscosity measured at a shear rate of 230 inverse seconds. Filtration was through a Dacron-wool filter. Viscosity measured at 37°C.

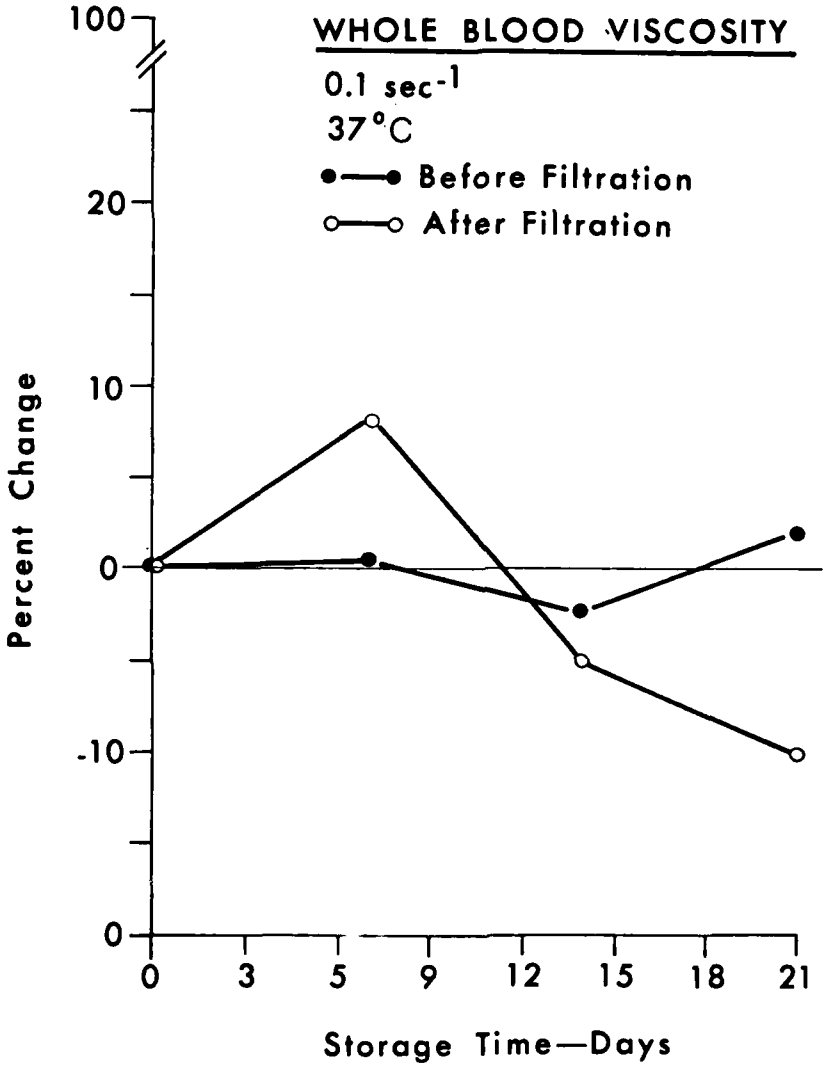

Fig. 2. Percent change during 21 days storage at 4°C of whole blood viscosity at a shear rate of 0.1 inverse seconds. Filtration was through a Dacron-wool filter. Viscosity measured at 37°C.

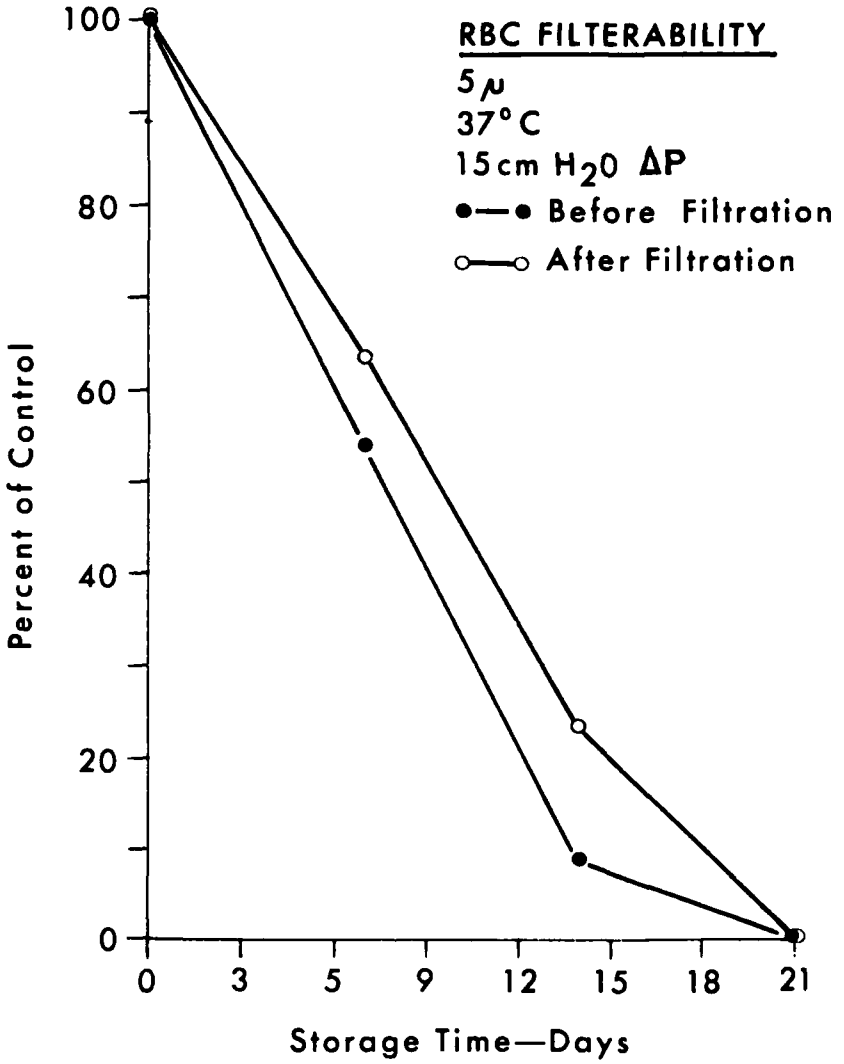

Fig. 3. Percent change during 21 days storage at 4°C of red blood cell filterability through 5μ polycarbonate sieves. Measured at 37°C with 15 cm H_2O driving pressure. Filtration refers to preliminary passage of whole blood through Dacron-wool filter.

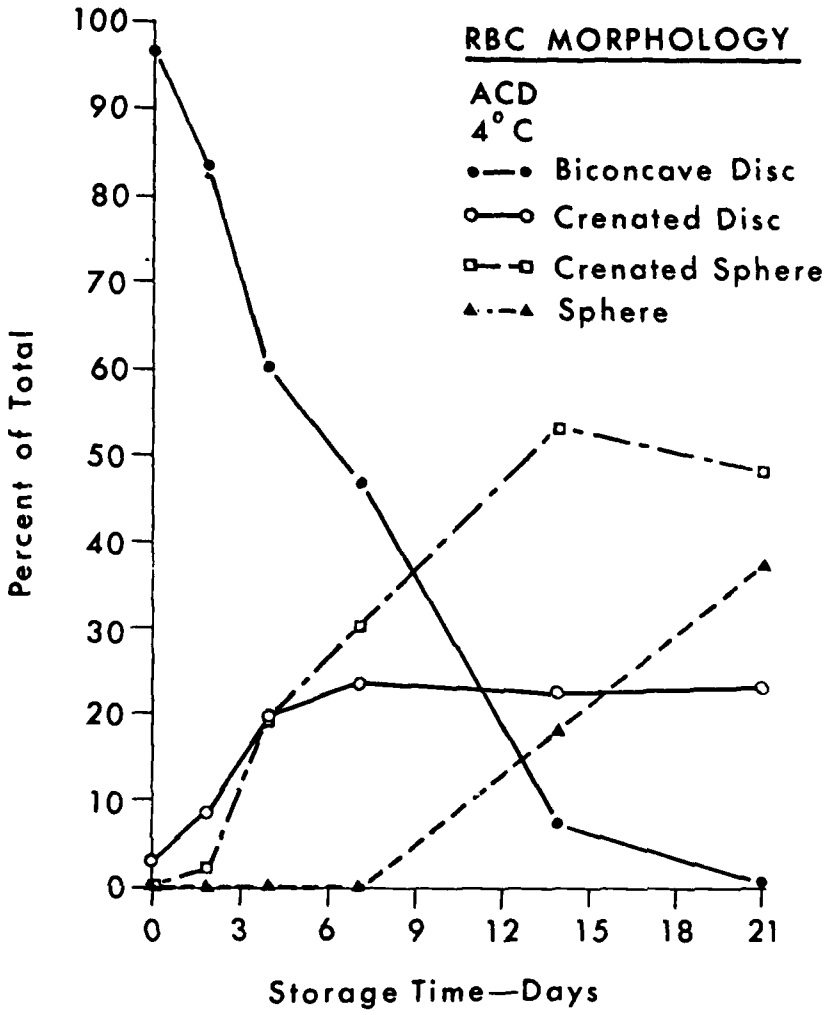

Fig. 4. Percent change during 21 days storage at 4°C of erythrocyte morphology. Blood preserved in ACD solution.

DISCUSSION

The changes in red cell shape noted in this study are in agreement with several others indicating a progressive disc-sphere transformation (13, 18). Progression from disc to sphere shape with storage has been correlated with depletion of erythrocyte content of high energy phosphate compounds, particularly adenosine-triphosphate (ATP) and it has been suggested that red cell shape and therefore deformability is dependent upon the relationship, at the cell membrane, between ATP, magnesium, and calcium (26, 27).

The changes in bulk viscosity and red cell filterability, as well as morphology, are analogous to those induced by acute exposure of fresh erythrocytes to hypertonic media. Normal biconcave discs have an excess area-to-volume ratio which is essential for normal deformability. The crenated and sphered cells have a lowered ratio of surface area to volume. They are therefore less deformable than normal erythrocytes. This accounts for the elevated viscosity at high shear rates, the changes in fluidity of concentrated (packed) red cells, and the decrease in filterability of the dilute red cell suspensions. These changes were progressive over the storage time of 21 days.

In contrast to the present studies, Weed, La Celle, and Merrill (27) found marked viscosity changes even at very low shear rates in blood only 24-30 hr old. Their study differed from ours in that no preservatives were used, the blood was defibrinated, and measurements were made at a hematocrit of 80%.

Current blood banking practice for stored blood required a minimum 24 hr *in vivo* survival of 70% of the transfused red cells. Blood stored in ACD for 21 days meets this criterion, and ACD has become the standard preservative solution (15, 25). As noted earlier, red cell shape and deformation are ATP dependent, and it is thought that the posttransfusion survival of stored erythrocytes is also related to cellular ATP levels in that the problem of survival of such cells relates directly to their ability to deform and pass through restricted regions of the microcirculation. Old and pathologic erythrocytes are less deformable than normal and their removal in the spleen and other reticuloendothelial tissues is thought to be a mechanical filtration process involving pores less than 5 μ in size. Weiss (28) has pointed out that these small channels in the splenic circulation may represent a relatively greater obstacle for stored red blood cells than for normal ones.

Blood is a precious resource. Because of fluctuating and unpredictable availability, continuous and increasing demands, and the necessity to maintain ample stockpiles for emergencies,

most transfusions consist of blood greater than 1 week old. By this time, severe abnormalities are already present in the cells. Because of its relative scarcity, efforts have been made to prol ng the storage life of banked blood. The use of citrate phosphate dextrose or CPD as the preservative prolongs the shelf life to 28 days (15). The present studies included a small number of units of blood collected in CPD and while there seems to be slight initial improvement in the variables compared with ACD, by 14 days the two are practically identical. The pH of CPD blood is higher than ACD preserved blood; therefore, hemoglobin function is better maintained in CPD due to the fact that 2,3 DPG maintenance is enhanced by high pH. The ATP maintenance, however, is favored by a lower pH (8, 9). Five millimolar adenine added to ACD blood promotes rapid regeneration of ATP and can extend the useful shelf life of such blood to 42 days (22). There do not seem to be any serious adverse effects of single unit transfusion of this material, and clinical trials of 8-9 units/patient suggest that it is safe. We have not studied any blood so treated.

There is obviously no currently available solution which is ideal for preservation of hemoglobin function as well as red cell membrane function, not to mention the hemostatic mechanisms. In some institutions, such as the San Francisco General Hospital, a walking donor program has been initiated for patients who are anticipated to require more than 10 units of blood in a short period of time in order to obviate these problems.

The syndrome of pulmonary insufficiency following shock and trauma is poorly understood. One of the etiologic factors is thought to be the infusion of large amounts of the amorphous material composed of platelets, white cells, fibrin strands, and other debris that accumulates in increasing amounts as banked blood ages (19, 23, 24). Several types of ultrapore filters have been developed which remove this cellular debris. The structure of these filtering devices is such that they offer a large surface to which particulate material will adhere upon making contact, so that the efficacy of filtration is determined not only by particle size but to a large degree by adhesiveness (12). The increased adhesiveness of platelets and white cells accounts for their impaired filtration and the lack of same accounts for poor erythrocyte removal.

Experimental and clinical studies have indicated that the proper use of ultrapore filters during transfusion markedly reduces the incidence of, but does not eliminate, pulmonary insufficiency (14, 21). The present study indicates that even removal of the microaggregate debris does not impart to the stored blood the flow properties it possessed prior to its donation.

In contrast to platelets and white blood cells, erythrocytes do not gain in adhesiveness upon storage and are, therefore, not removed by ultrapore filters *in vitro*. They are, however, removed *in vivo*. Severely damaged red cells are removed promptly and less severely damaged ones are removed during the first 24 hr after transfusion.

The lung is the first microvascular bed encountered by intravenously administered blood cells. Quite apart from the problem of erythrocyte destruction and removal, recent studies on the pulmonary circulation indicate that alterations in whole blood viscosity and red cell flexibility can significantly alter pressure-flow relationships, especially by increasing pulmonary vascular resistance (11), and perhaps by increasing pulmonary capillary permeability (3). Furthermore, it has been shown that the pulmonary circulation is impaired by crenated red cells (10), and we have observed impaired microvascular flow due to red cells with decreased deformability in experimental microvascular beds.

McNamara *et al*. (19) have observed a significant stepwise reduction in screen filtration pressure in samples from central venous to arterial to femoral venous blood. This suggests that the infused material is being retained primarily by the pulmonary and secondarily by the peripheral vascular bed. Studies such as these have led to the concept of the lung as an "*in vivo* filter."

The present results do not suggest that the stiff red cells of banked blood produce widespread microvascular occlusion, but only that administration of large volumes of these rheologically abnormal cells, which are not removed by ultrapore filtration, can produce circulatory disturbances, primarily in the pulmonary circulation, which are disadvantageous to the recipient of massive transfusion at a time when his/her compensatory mechanisms are severely limited by the events which necessitated the transfusions, and it may, therefore, be a contributory factor in the development of the adult respiratory distress syndrome of shock and trauma.

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SESSION II

June 21, 1977

Letterman Army Institute of Research

San Francisco, California

MICROEMBOLI SIZE AND TISSUE PATHOPHYSIOLOGY

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The increasing use and acceptance of microfiltration of blood during transfusions and extra corporeal circulatory assists has left unsolved many problems. Some of these concern the chemical causes and changes related to platelet-leukocyte aggregation. Others are of practical clinical importance, one of which concerns the number and size of microemboli which will cause significant pathophysiological changes. It is to this problem of microemboli size that I will address myself.

Two polarized points of view can be identified. The one opines that only the larger microemboli, those of a diameter of 40 μ or greater, are sufficiently harmful to make necessary their removal. At the opposite pole are those who believe that platelet-leukocyte aggregates of all sizes are potentially toxic and, therefore, should be removed. There are also intermediate points of view. It seems agreed that the larger aggregates can be harmful and should not be infused into patients. I will attempt to convince you that the smaller aggregates, down to and below 10 μ in diameter, are also harmful and should be removed.

Let us consider first some earlier studies of the occlusive effects of noncompressible microemboli, i.e., plastic or glass beads, seeds, etc. This subject was reviewed in 1950 in Physiological Reviews by Whitteridge (1). He noted that intravenous injection of microemboli (those 100 μ in diameter or smaller) were attended by tachypnea, increased pulmonary artery pressure, and reduced arterial pO_2. The occlusion of a main pulmonary artery by ligation, or by macroembolism did not cause these or other obvious physiological changes, nor did injection of the minute emboli if the pulmonary artery into which they were injected was immediately occluded. In comparative studies of different sized microemboli the smallest, those in the 10-μ range, were found to be severely toxic. The tachypnea could be abolished by deep anesthesia. The microemboli caused vascular engorgement, edema, and eventually capillary hemorrhages.

Subsequently in 1954, Swank and Hain (2) injected paraffin emboli labeled with lamp black of mixed sizes ranging from 4 to 12, to 15 and to 17 μ in diameter into the Cerebral circulation of anesthetized dogs. Immediately, tachypnea, a marked fall in systemic blood pressure, and abolition of the electrical activity of the brain resulted with a subsequent gradual recovery to

normal. Most of these emboli cleared the brain in 10 min, but some of the 14-, 15-, and 17-μ emboli were still to be seen histologically 6 and 24 hr after the injection. Histological studies showed a marked increase in permeability of the blood-brain barrier by trypan blue, and destructive lesions primarily in the white matter and junctional area between the gray and white matter. Also, passage of the very small emboli resulted in an increased adhesiveness of the endothelium so that small granules of injected lamp black came to lodge on the endothelial surfaces, outlining the microcirculation. The lamp black did not adhere to normal nonembolized endothelium.

More recently, the vasculature of the lung was studied with rigid microspheres of uniform size by Ring et al. (3) and Kaihara et al. (4). All spheres less than 2 μ in diameter passed through the microcirculation of the lungs during a single circulation time. About 20% of those 2.8 - 4.0 μ in diameter, 70% or more of those 5.7 - 8.0 μ in diameter, and about 95% of those 8 μ in diameter were retained in the lungs. Almost all of those 15 μ in diameter and all of those 50 μ in diameter were retained for one circulation time or longer. In the subsequent 5 - 10 min only about 1% of the 15-μ spheres negotiated the lungs; the remaining 99% remained trapped. In other studies 15-, 50-, and 80-μ spheres were injected into the left heart and 3 - 10 min later they were looked for in the various organs. The 50- and 80-μ spheres were completely trapped by the various organs, but 5 - 10% of the 15-μ particles negotiated the peripheral capillary beds and were collected in the lungs. After similar injections intravenously almost 100% of the 15-μ spheres were trapped and held in the lungs, none having traversed this vascular bed in the 10-min observation period. During the subsequent 15 days no reductions in the 50-μ microemboli in the lungs occurred, although it was observed in the study referred to above that a significant number of 30-μ paraffin-lamp black emboli did negotiate the microcirculation of the brain after delays of days or weeks.

Detailed anatomical studies of lung components revealed measurements consistent with the solid-sphere studies just mentioned. Weibel and Gomez (5) found that there are about 300 million alveoli in the human lungs with an equal number of precapillaries with internal diameters averaging about 20 μ (15 - 25 μ). From these precapillaries arise many more capillaries which are interrupted at frequent intervals by interconnecting capillary segments. The capillaries have an average internal diameter of 8.3 μ. Actual internal diameters are far from uniform as shown by the solid-sphere studies which indicate that the "functional" internal diameters vary from 3 to 9 μ (3).

It may be of some concern how normal red cells with a diameter of 7 - 8 μ quickly negotiate lung capillaries, many of which are less than half their size. Two mechanisms are possible and have been suggested. If the pulmonary capillaries are oval (perhaps 8 μ by 3 μ) the small diameter would restrict the passage of spheres, but not the red cells which have a thickness of 2 μ. If the capillaries are round, the red cells could deform to accomodate for and negotiate these constricted small round capillaries without undue delay. Normal human red cells are very pliable and have been shown by Jay (6) to easily traverse round capillary tubes of about 2.9 μ inner diameter without alteration in their surface area or volume. The critical squeeze is through a capillary tube 2.5 μ in diameter which results in irreparable damage to the red cells.

These observations lead to several tentative conclusions. First, a large proportion of solid spheres less than half the average internal diameter of capillaries are delayed in their passage through the lungs and other organs; second, after delays of hours or days solid spheres several times larger than the diameter of capillaries will work their way through capillary networks; and third, the capillary bed of the lung is more restrictive, and sequesters more small emboli than other capillary beds of the body. One can add that the passage of very small emboli (those 15 μ and less in diameter) through the capillary bed of the brain increases the permeability of the blood-brain barrier, and the adhesiveness of the endothelial lining of the microcirculation, and that passage of very small microspheres through the microcirculation of the lung causes physiological changes consisting of tachypnea, pulmonary hypertension, general arterial hypotension, and a reduced arterial pO_2.

However, we are not concerned with solid-sphere microemboli, but with somewhat pliable microemboli of biological origin made up primarily of platelets and leukocytes plus variable amounts of undefined debris. Two important sources of these embolic can be defined, the one formed in vitro during storage of blood (25), and the other formed in vivo, for example, as the result of surgical shock (26), or during the use of an extracorporeal circulatory assist. The in vitro formed emboli are probably slightly pliable or deformable. From Solis and Gibbs' (7) early work some idea of the degree of deformability can be gained. The passage of outdated blood through a sieve-type filter with multiple pores 40 μ x 40 μ square (Pall filter) completely removed those from 50 to 100 μ in diameter. Aggregates less than 50 μ in diameter readily passed through the pores of the 40 μ filter. Deformability and/or fragmentation would appear to have occurred to those aggregates ranging in diameter from 50 to 100 μ. From numerous screen filtration pressure measurements

with screen having multiple pores 20 μ x 20 μ square very little evidence of deformability of *in vitro* formed aggregates was observed. The screen filtration pressure curve had no tendency to level off as it would if a substantial number of emboli were forced through the 20 μ x 20 μ pores. Instead the rise in screen filtration pressure (SFP) curve accelerated even as the driving pressure rose to 400 mm Hg or more.

When dealing with *in vivo* formed microemboli there is reason to expect a greater degree of deformability, and this is shown by the screen filtration pressure curves. The curves tend to level off between 200 and 400 mm Hg and exhibit a saw-toothed configuration, suggesting a balance between the addition of aggregates to the screen from passing blood and the removal of aggregate material by extrusion through the pores of the screen. However, in neither case does it seem likely that the deformability is as great nor as consistently present as in the red blood cell.

Let us now examine some of the evidence available from animal and human studies. Jenevein and Weiss (8) using light microscopy observed microemboli in small arterioles and capillaries after massive whole blood transfusions without microfiltration during open heart surgery. Most of these vessels are approximately 20 μ in diameter or smaller. Similar observations were made by others (9,10,11).

With the electron microscope (EM) it is possible to more clearly define the aggregates and delineate the damage produced by these emboli. Connell and Swank (12) studied lungs and other tissues immediately after transfusions and for hours and days thereafter. Almost all microemboli were observed obstructing precapillaries, capillaries, and venules. None were observed in arteries. They almost all ranged from 10 to 20 μ in diameter. One must not forget, however, that with the electron microscope one can analyze only very small areas even when numerous samples are studies. It is, therefore, possible that a few larger microemboli could have been missed. On the other hand, larger emboli could have fragmented in the arteries and arterioles where the hydraulic and shearing forces are relatively high. The resulting smaller particles could then come to rest in the smaller vessels downstream where the pressure and speed of flow are substantially reduced and the endothelia would be in more intimate contact with the adhesive aggregates.

In addition to the occlusion of the microcirculation, platelet-leukocyte emboli also caused erosion of the endothelium, interstitial tissues, and finally the alveolar

epithelium allowing the vascular contents to flow into the alveoli. The beginning of erosion of the endothelium was observed within 20 min of the end of transfusion, and scarring was present for 2 weeks in several animals.

In our original EM studies (12), the transfused blood was 5 - 7 days old. In subsequent studies, with blood stored 10 - 14 days, many fewer and less severe lesions were found, suggesting neutralization or exhaustion of the lytic enzymes which we presumed were released from the platelets and leukocytes forming the aggregates.

It is well known that massive transfusions increase the pulmonary artery pressure and alter blood gases in both animals and man (10,13-17), and increase vascular resistance in other microcirculatory beds (18). However, the studies of Litwin et al. (17,19) also throw some light on the relationship of emboli size and pathophysiologic changes. In one study they investigated pulmonary artery pressure, arterial pO_2, pCO_2 and pH, and total body consumption of oxygen after infusion of old high screen filtration blood through no filter, a standard commercial clot filter, and the Swank microemboli filter (17). These parameters remained normal after removal of most of the small as well as large microemboli by the Swank filter. Marked abnormalities resulted after no filtration and after filtration through a standard clot filter, although some benefit was observed from use of the standard clot filter which removes some of the large emboli. In another study (19), these workers compared biochemical parameters after removal of most emboli of all sizes by the Swank filter, and removal of most of the emboli 50 μ and larger in diameter but only a few emboli smaller than this by the Pall filter. The pH of arterial blood and total oxygen consumption decreased, arterial pyruvate and lactate levels increased, the precentage of arteriovenous shunting (qs/qt) increased, and pulmonary diffusing capacity for O_2 (DO_2) decreased after transfusions through the 40-μ screen (Pall filter). These parameters remained normal when the Swank filter was used. In all instances postinfusion differences between the Pall and Swank filters were significant.

These studies demonstrate that removal of large emboli down to 50 μ in diameter was definitely very beneficial to the recipient even though the physiological parameters became abnormal. Efficient filtration, with removal of most of the small as well as large emboli, however, benefited the recipients even more and to the extent that the physiological parameters remained unchanged after the transfusion. They also demonstrate that the toxic effects of the smaller microemboli (those

smaller than 50 μ in diameter) were probably related to embolization of tissues other than the lungs. In a previous study such secondary embolism after transfusion has been confirmed anatomically (23).

Haggart and Walker (20) produced graded occlusion of the left main pulmonary artery or of the pulmonary artery itself. They found no significant variation in the general physiological condition of the animals until 52 - 66% of the cross-sectional area was occluded. Later extension of this work by Gibbon et al. (2) showed that occlusion to 60% was without effect on arterial or venous pressures but between 60 and 85% obstruction was. At that point cardiac output and arterial pressure were reduced. Hyland et al. (22) studied the effects of graded emboli of uniform sizes, to match selectively the pulmonary arteries from lobar arteries down to atrial arteries 170 μ in diameter. With each size a majority of vessels had to be occluded before pulmonary hypertension occurred with or without anesthesia. In only a few cases did tachypnea result. This may have been due to the fact that the more toxic microemboli below 100 μ in diameter were not studied.

Thus it would appear that even slight evidence of pulmonary insufficiency from vascular occlusion would indicate that approximately half or even more of the pulmonary vasculature was occluded in healthy individuals. If we are dealing with elderly individuals with reduced lung capacity and presumed reduced microcirculatory bed, the matter of occlusion of the microcirculation during transfusions becomes very important.

One last point concerns the enlargement of very small in vitro-formed emboli after introduction into a patient. Is it possible that the recipient's "normal" platelets and leukocytes contact and then adhere to these adhesive masses of cells thereby enlarging them? Some recent observations by Connell et al. (24) suggests that this may occur. In studies of human lung biopsies a short time after intravenous infusion of platelet packs many platelet microemboli were observed in the microcirculation. Immediately after infusion many smaller blood vessels contained platelets alone attached to the endothelium. Noteworthy, however, is the fact that about 1 hr later all aggregates contained polymorphonuclear leukocytes. (This is what Connell and myself observed after production of acute hypotensive shock (27). Immediately the blood vessels contained masses of platelets. An hour or more later, leukocytes in large numbers were present and the platelets were in the process of advanced dissolution.) These appear to have become incorporated into the platelet aggregates introduced into

the body only minutes before. These observations would suggest that leukocytes of the recipient patient, and perhaps also platelets, do in fact become incorporated into, or added to platelet or platelet-leukocyte aggregates infused into the body.

The evidence suggests that small platelet-leukocyte microemboli (20 μ or less in diameter), as well as the larger ones, are harmful when they are infused in large numbers. Our information is incomplete, however, and must await direct data, both clinical and experimental, in which microemboli of selected sizes are infused, and the pathology and physiology are observed.

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EVOLUTION OF INTRAVASCULAR AGGREGATION IN ACUTE SURGICAL ILLNESS

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Erythrocyte Aggregation

The subject of intravascular aggregation is not new to students of trauma. In 1945, Knisely et al. (23) documented the aggregation of red cells within several hours of injury. In 1947, he published his observations of intravascular red cell aggregation in a multitude of conditions and established an association between intravascular aggregation and coronary artery disease (22). Loss of capillary integrity was observed to occur as a consequence of intravascular aggregation, evidenced by extravasation of plasma from the vascular space, accompanied by packing of red cells within vessels. Red cell aggregation and human blood rheology have since been studied by a number of investigators. Quite recently, Scholz et al. (38) showed elevation in plasma viscosity with surgical procedures and Litwin et al. (17) suggested that intravascular aggregation after surgical trauma may cause circulatory impairment, as evidenced by decrease in total body oxygen consumption. Resolution of intravascular aggregates in Litwin's studies followed administration of low molecular weight dextran. While mechanisms for red cell aggregation are not completely resolved, there appears to be a relationship to alteration of plasma proteins, especially fibrinogen. One attractive concept is the occurrence of alteration of erythrocyte surface charge, since liberation of sialic acid from the coating protein of red cells can cause cellular aggregation by altering red cell electronegativity, hence increasing intercellular attractions. Enzymes that liberate sialic acid such as papain, trypsin or neuraminadase are examples of substances capable of diminishing surface charge and increasing aggregation. Certain bacteria and viruses possess enzymes similar to neuraminadase, suggesting that this mechanism may contribute to microaggregation identified in the context of infection (10). Administration of alimentary or intravenous fat to rabbits also causes intravascular aggregation of red cells (11) and others have demonstrated platelet aggregation with the administration of intravenous fats. This observation is important, for trauma is frequently associated with release of fats into the circulation.

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Platelet Aggregation

Intravascular aggregation of blood platelets has also been studied quite intensively. Like red cell aggregation, altered platelet adhesiveness has been documented in association with a number of pathologic conditions, including shock, trauma and cancer, either as causative or coexisting factors. Platelets are remarkable blood elements which, while of short physiologic life (several days), have the ability to form intravascular aggregates which may also initiate the coagulation process. Coagulation at a focus of intimal injury is initiated by the well-studied platelet plug, which occurs by adherence of blood platelets either to capillary endothelium or to collagen. Adenosine diphosphate (ADP) is the principal mediating agent in the ensuing aggregation process, and platelet aggregation may be perpetuated by the further release of ADP from aggregating platelets themselves. Ultimately, fibrin deposition and a firm hemostatic plug result at the site of injury. Decreasing platelet function and size are associated with increasing platelet age (21). Large, young platelets are mobilized early and are the first removed during extracorporeal circulation. This phenomenon is probably responsible for reports of plugging of arterial microfilters by aggregate material at the onset of bypass and for altered platelet reactivity that occurs with cardiopulmonary bypass (3,24). Neutropenia also occurs with extracorporeal circulation accompanying hemodialysis, due to sequestration of large numbers of neutrophils in the lungs during the first few minutes of dialysis (41). This reaction may have a parallel in the leukopenic and thrombocytopenic reaction to infusion of pyrogenic materials and colloidal suspensions. While many believe that platelets or their release products can produce endothelial damage, platelets may also be supportive of capillary integrity (42). Administration of platelets in isolated organ perfusion has been associated in some instances with enhanced organ preservation, yet experimental homograft rejections is associated with platelet rather than red cell aggregation (14). Although platelet and erythrocyte aggregation may often occur in the same settings, there is evidence to suggest that platelet aggregation occurs first (2) and that platelet aggregation may have the greater effect upon capillary integrity and subsequent organ dysfunction.

Occurrence of Intravascular Aggregates

In the normal organism, intravascular microaggregates are probably forming and dissolving continuously and it is indeed remarkable, that blood usually remains in a continuously fluid state. At sites of vascular branching and other turbulent foci, basic flow factors (e.g., venous vs. arterial) and the existence

of vascular branching or intimal damage are also important. Vibratory frequencies of low acoustic energy occur at sites of vascular branching which are capable of initiating aggregation and perhaps stimulating atherosclerosis (31,34). Fortunately, there is a wide margin between the formation of platelet aggregates and the formation of fibrin thrombi in vessels. In some pathologic states, intravascular aggregation is almost assured and its occurrence has been well documented. Sepsis is a classic example and experimental administration of endotoxin readily produces platelet aggregation (27). Soft tissue injury results in release of fragments of collagen, fat and tissue thromboplastin into the blood stream, all of which are potent activators of the coagulation system. Robb (35,36) has documented emboli originating from the microvasculature of the bowel wall with hemorrhage and endotoxin, and from injured areas after trauma. Swank (39) showed that trauma and transfusion in animals are also accompanied by an increase in platelet aggregation, as measured by screen filtration pressure (SFP). Screen filtration pressure involves the passage of blood at a constant rate through a 20 μ filter. Sidearm pressure proximal to the filter has been shown to be proportional to platelet aggregation (40). Even gentle, localized trauma may be followed by blood platelet aggregation and distant embolization, the extent of aggregation and embolization probably being proportional to the severity of the injury (27).

Stimulated by Swank's suggestion that intravascular aggregation with trauma may contribute to clinical pulmonary insufficiency after injury, we studied the changes in screen filtration pressure in blood from a group of 89 moderately to severely injured combat casualties in Vietnam (5). Screen filtration pressure was measured serially in arterial and venous blood beginning immediately after the arrival of the patient for treatment, before the administration of bank blood and often before administration of any intravenous fluids. Multiple interchangeable SFP chambers allowed the rapid determination of multiple samples. In this group of injured soldiers, 58.9% of patients had elevation of arterial and/or venous SFP at the time of admission. Increased SFP on admission was accompanied by base deficit in 53.6% of patients. Although significant SFP elevation rarely was observed immediately after massive transfusion, SFP elevation in such patients frequently occurred on succeeding days. These studies suggested that platelet aggregation was occurring as a primary intrinsic response to injury. Of equal importance was the observation that when both arterial and venous SFP were elevated at the time of admission, arterial hypoxemia during the succeeding 4 days was almost assured. When patients with chest injuries were excluded, 95% of patients with elevation of admission SFP in both arterial and venous blood became hypoxemic during the

succeeding 4 days, whereas only 50% of a similar group of patients without SFP elevation became hypoxemic during this time.

While many investigators continued to explore the possible relationship between aggregate infusion and subsequent occurrence of pulmonary insufficiency, we have extended our patient studies to the animal laboratory and measured the same parameters in a group of baboons subjected to limited soft tissue hind-limb trauma with a captive bolt pistol, with or without concomitant arterial ligation, but without fluid resuscitation (9). In these 19 baboons, transient increase in limb-venous SFP was uniformly observed after trauma. Mean increase in SFP was 104.1±20, returning to normal after 10 min. The addition of femoral fracture in three baboons increased SFP in the venous effluent of injury still further. Yet common femoral arterial ligation produced no greater change in SFP than in animals without such vascular interruption. Changes in SFP were accompanied by no significant alteration in blood pressure, pulse rate or arterial oxygen tension. Respiratory rate and minute volume were controlled by mechanical ventilation. These experiments confirmed the suggestion that trauma is associated with the liberation of a shower of aggregate material into the venous circulation. Slight reduction in platelet count, the finding of granular material on microscopic examination of the SFP screens and increased lung uptake of radioactive platelet label in these animals suggested that platelets were responsible for these aggregates. Subsequent experiments were performed in baboons made thrombocytopenic by prior administration of baboon-specific platelet antiserum. Elevation of screen filtration pressure in the venous effluent of injury in these animals did not occur, lending further support to the concept that platelet embolization had been occurring in the injured animal with normal platelets. Studies of Ljungqvist and others (28) using radioactive-labelled platelets have supported the concept of pulmonary platelet microembolism with trauma. In addition, Modig *et al.* (32) showed the pulmonary appearance of radioactive-labelled fibrinogen soon after intraoperative impaction of a femoral prosthesis, accompanied by diminution in arterial oxygen tension.

Reduction in blood platelet count, which may occur in the context of hemorrhage and major trauma, suggest that trapping of platelet aggregates may be occurring elsewhere in the circulation. Both the lung and the spleen are capable of platelet sequestration and both of these organs can release platelets into the venous circulation with the injection of adrenalin (27). Rebound thrombocytosis which occurs after hemorrhage, trauma and surgical procedures may also be related

to release of megakaryocytes from bone marrow, each of which is capable of producing 2,000 - 6,000 platelets. The occurrence of increased platelet aggregation and adhesiveness after hemorrhage uncomplicated by trauma has been documented by some and refuted by others. Our studies (4) concur with those of Ljungqvist and Schwartz (29), that platelet aggregation and microembolism do occur with hemorrhage. In addition, Eriksson et al. (18) have demonstrated increased adherence of leukocytes to venules after 30 ml/kg hemorrhage in rats. This effect is worsened with heparin therapy, unaffected by low molecular weight dextran or saline but improved by corticosteroids. In view of the enormous capacity of the reticuloendothelial system, aggregation which may occur with pure hemorrhage is probably only transient, unless there is simultaneous inhibition of the fibrinolytic system. Unfortunately, the association between microaggregation and impaired fibrinolysis may not be that uncommon (26). If hemorrhage is superimposed upon other factors (e.g., trauma, sepsis, burn injury, long bone fracture) both aggregation and embolization are likely to be enhanced.

Mechanisms of Intravascular Aggregation

Mechanisms for the initiation of intravascular aggregation after shock and trauma may relate in part to sludging, acidosis and hypoxemia within the microcirculation. Blaisdell and Shlobohm (12) have drawn a parallel between hypoperfusion with "washout" of ischemic tissue following release of peripheral vascular occlusion and the process of aggregation and microembolism that may occur after correction of peripheral blood flow due to other causes, such as hemorrhage. Contact of blood elements with injured capillary basement membrane and release of tissue thromboplastic substances, fats, catecholamines and platelet or red cell adenosine diphosphate have all been implicated in the pathophysiology of this phenomenon, since all of these elements can induce platelet aggregation. More speculative elements that may contribute to intravascular aggregation include serotonin, histamine and lysosomal enzymes (44), all of which may be found in platelets and may alter either vascular reactivity or the integrity of capillary membranes (16), with subsequent leakage from the intravascular space (25,33). In addition to these humoral factors, this permeability response may be mediated in part by the sympathetic nervous system (13).

Intravascular aggregation probably occurs as a primary adaptive phenomenon, initiated by contact between platelets and injured capillary endothelium. Contact of blood platelets with collagen is, indeed, one of the basic mechanisms for initiation of platelet aggregation in order to bring about cessation of bleeding. For this reason, we postulated that the connective

tissue-platelet reaction may be fundamental in the evolution of intravascular aggregation with shock and trauma. Since alteration in composition of connective tissue has been described with shock and trauma, we designed an experiment to study the hypothesis that connective tissue from injured animals may have a different capacity for initiating platelet aggregation than connective tissue of uninjured animals. In these experiments (4), 30 rats were subjected to hemorrhage (2.1% body wt) and 30 to burn injury (20% upper dorsal scald) and intravascular aggregation was measured by screen filtration pressure. Prior to sacrifice, rat tails were removed for preparation of connective tissue homogenates. These homogenates were then incubated with normal human platelets in an aggregometer. Increased screen filtration pressure was observed in these animals both after hemorrhage and burn injury. In addition, connective tissue obtained 2 hr after burn injury produced platelet aggregation with 38.8% greater slope and 56.4% larger aggregates than connective tissue from uninjured controls. Connective tissue obtained after hemorrhage when incubated with normal human platelets produced aggregates with 43.4% greater slope and 40% larger aggregates than connective tissue from uninjured controls. These data suggested a systemic effect of shock and trauma in terms of an accelerated ability of connective tissue to aggregate blood platelets. This factor may be in part protective and may be additive to the state of altered platelet reactivity which often occurs in the clinical settings of shock, trauma, and surgical operations.

Fortunately, intravascular aggregation of platelets is usually reversible and aggregate material is rapidly consumed by the reticuloendothelial system. Of course, tranfused blood is also rich in microaggregates, principally composed of dead or disintegrating platelets and leukocytes. This nonfunctional cellular debris may present a large load of particulate matter to the venous circulation unless the transfused blood is filtered. In addition to this obvious extrinsic factor in transfused blood, elevated levels of adenosine diphosphate in stored blood may be observed which may contribute to microaggregation in the recipient circulation. We have studied platelets and whole blood during storage at $4^{\circ}C$ and have shown that when platelet concentrates are allowed to age, their screen filtration pressure is equivalent to that of stored whole blood. When these stored platelets are then added to fresh blood, aggregation (SFP elevation) is much greater than when ADP alone is added to fresh blood. When both ADP and stored platelets are added to fresh blood, SFP is approximately 100 mm greater than the predicted sum of ADP and platelet addition to fresh blood. These facts suggest the possibility of an interaction between stored platelets and fresh platelets (or red cells) in

the recipient circulation. These observations suggest that in vivo aggregation of blood elements with shock and trauma may be accentuated by the administration of aging blood, independent of considerations regarding the load of particulate matter that is administered (8). Obviously, particulate matter is also important, for arterial transfusion of stored blood results in a higher incidence of renal tubular necrosis than conventional intravenous transfusion (20).

Although the list of factors that may be brought to bear upon intravascular aggregation is obviously large, one additional element may warrant mention. Since hormonal factors are so frequently evoked in the context of in vivo aggregation, we have studied intravascular aggregation in an animal model known to be associated with endogenous hormonal excess. Since increased thrombus formation sometimes occurs with pregnancy and parturition, intravascular aggregation was studied in pregnant and nonpregnant (male and female) rats. These animals were studied before and after administration of 2 cc intraperitoneal hypertonic saline (an abortifacient when administered intrauterine). Pregnant rats were studied in the second trimester of pregnancy. Only pregnant animals showed any evidence of in vivo aggregation after administration of hypertonic saline. Two hours after saline infusion, pregnant animals showed 47.6% diminution in fibrinogen, 47.8% diminution in platelet count and 166.7% increase in SFP, accompanied by increased splenic and pulmonary uptake of radioactive platelet label (7). These effects did not occur after administration of other agents of equal osmotic or ionic effect (urea, lithium chloride), or in males or nonpregnant females. Under normal circumstances these observations would be dismissed as an idiosyncracy of the gravid state, since pregnancy is often considered a state of subclinical intravascular coagulation. However, the curious feature of these studies is that offspring of pregnant animals which had been administered intraperitoneal saline showen significant diminution in pulmonary surfactant (amniotic lecithin/sphingomyelin ratio) and diminished pulmonary expansion, compared with untreated controls (7).

In summary, in vivo aggregation of blood elements probably occurs with regularity in patients with hemorrhage and trauma and in a number of other clinical entities. This phenomenon is usually subclinical (i.e., detected principally by blood studies) and related to multiple factors. The nature and the extent of injury and associated hypovolemia and hemoconcentration are probably critical elements in determining its occurrence. In addition, the association of fat embolism with soft tissue injury as well as with skeletal trauma is recognized and chylomicra are frequently found in combination with intravascular platelet aggregates (2). Fatty acids also stimulate platelet aggregation (30) and the fact that pulmonary fat embolism in

injured animals occurs in synchrony with the appearance of intravascular white masses elsewhere in the circulation (2) suggests that disturbance in the equilibrium of fat in the blood may have a similar etiology to disturbance of blood suspension stability which may contribute to microaggregation (19). Hence, fragmentation of tissues following trauma may allow access of fatty elements as well as platelet aggregates to the venous side of the circulation. In fact, Collins et al. (15) have suggested that inapparent hypoxemia in combat casualties may sometimes represent a form of subclinical pulmonary fat embolism. Sludging of red cells in injured tissues provides an excellent nidus for interaction of red cells with blood platelets, an acceleration of aggregation (sometimes with thrombus formation) and distant embolization. Of course, the effects of all of these factors may be worsened in some patients due to post-traumatic enhancement of coagulability and depression of the reticuloendothelial system (37).

Significance

Estimation of the clinical significance of these factors is one of the objectives of this symposium. Multiple organ dysfunction secondary to embolization of intravascular aggregates has been postulated by numerous investigators. The presumed origin of aggregates in the slower flowing venous side of the circulation, the direct access of such material to the filtering action of the pulmonary circulation and increased recognition of clinical pulmonary dysfunction after injury provide wide avenues for pathophysiologic speculation. Although aggregates have been demonstrated in pulmonary arteries of patients dying after trauma, experimental support for a specific pulmonary lesion secondary to post-traumatic intravascular aggregation has been variable. The problem is complicated by several paradoxes. Anatomically, pulmonary intravascular aggregates are often difficult to define, even with infusion of adenosine diphosphate into the central circulation. Physiologically, the effects of intravascular aggregates may be more likely to produce hyperventilation than hypoxemia. Probably, the effect of aggregate material upon the lung is related to factors other than simple mechanical plugging of pulmonary capillaries. The capacity of platelet substances (e.g., histamine, serotonin) for altering vascular reactivity and permeability and for producing bronchiolar constriction, make this a reasonable hypothesis. Perhaps, as Hechtman et al. (43) have suggested, platelet-lung interaction occurs at sites of endothelial injury and this interaction serves to optimize regional ventilation-perfusion by redistribution of blood flow and ventilation away from damaged lung units. In later stages, these protective mechanisms may fail, resulting in extensive

lung damage and obvious pulmonary insufficiency. Since platelets are capable of altering capillary permeability and since pulmonary interstitial edema is a common observation in clinical and experimental respiratory distress, we have studied pulmonary permeability to Evans Blue dye (4) with hemorrhage and burn injury and with transfusion of stored blood and platelets (6). Pulmonary permeability is increased in each of these experimental models and radioactive platelet label appears in lungs and spleen. These findings support the suggestion that platelet-lung interaction may lead to endothelial damage, encouraging the development of pulmonary interstital edema, lowering of functional residual capacity and diminishing lung compliance (43).

SUMMARY AND CONCLUSIONS

Intravascular microaggregation which occurs in acute surgical illness has a basic foundation in fundamental homeostatic mechanisms principally related to alteration in reactivity of circulating blood platelets. The effects of intravascular aggregation may be accentuated by the extent of injury, limited cardiopulmonary reserve, therapeutic maneuvers (including stored blood) and postoperative complications (especially sepsis). As with many conditions in which treatment is prompt, intravascular aggregation is usually reversible once the etiologic mechanism is aborted. Hence, its sequellae are usually subtle if at all measureable. Most likely, there is a synergism between the significance and extent of injury, the need for massive transfusion and the occurrence of sepsis which makes the syndrome relevant in only a small percentage of patients. Hormonal factors may play a role in intravascular aggregation and its effects. Endothelial damage with transcapillary leakage of fluid and protein may represent the final common pathway in organ dysfunction resulting from microaggregation.

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SCREEN FILTRATION PRESSURE AND ITS CLINICAL SIGNIFICANCE

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Screen filtration pressure (SPF) is a tool for estimating the quantity of microaggregate particles in stored blood. As we have previously noted, stored blood develops large volumes of microaggregate material composed primarily of platelets, fibrin, and leukocytes. This material accumulates in progressively larger quantities as the period of storage increases.

SFP, first described by Swank (1), measures the resistance to flow of blood through a 20 μ mesh screen filter. As microaggregates accumulate in stored blood, the resistance to flow and consequently the SFP increases progressively.

Interest in microaggregate formation in blood has since extended to include studies on microaggregate formation following shock and trauma and during cardiac pulmonary bypass. Screen filtration pressure is the technique most commonly used in studying microaggregate formation in each of these clinical situations and its significance in this regard is the topic of the current report.

Evaluation of the Method.

At best, SFP is a semiquantitative method of estimating microaggregate quantity. The absolute value of SFP, assuming a constant screen size, depends not only on the quantity of microaggregate material but on the rate of blood flow through the apparatus, the volume of blood infused, and the surface area of the screen exposed. Other factors which influence blood viscosity will also influence SFP including hematocrit, fibrinogen concentration, and temperature of the blood (2).

Furthermore, in blood stored 2-3 weeks, microaggregate size increases sufficiently so that even a single aggregate can cover the surface area of the screen and variations in SFP determinations within the same sample increase as the blood ages (3).

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Using most standard techniques for determining SFP, stored blood SFP exceeds 500 mm Hg within a few days and transducers with higher pressure capabilities are essential. If a slower flow rate is used, then a smaller, less representative sample is exposed over the same period of time. In any case, it is evident that, using SFP, it is difficult to establish a universal method resulting in comparable baseline values. Consequently, new control values must be established each time a new experiment is initiated and comparison of absolute values between different studies is of no value.

We have reported a modified method of measuring microaggregates using the SFP apparatus and weighing the screen sections before and after the passage of blood through the apparatus (3). This gave a direct measurement of debris weight per cubic centimeter of blood. The method proved more time-consuming and with less reproducibility than the standard SFP method which was particularly pronounced as the blood aged. We have consequently abandoned debris weight as a method of quantitating SFP.

Evaluation of SFP by Other Methodologies.

Histological: The increase in SFP in stored blood is accompanied by an increase in platelet microaggregates seen on smears of whole blood and platelet-rich plasma from stored blood (Fig. 1). As SFP increases, both the number and size of these particles increase (4-6).

Electronic particle counting: Temporal evolution of SFP and debris volume determined by an electronic particle counter are very similar (Figs. 2a and 2b). The apparent decrease in debris volume, noted here, reflects the progression of some microaggregate particles to a spherical diameter of >80 μ and they are not counted by the present method. In fact, visible debris is frequently present in the blood at that point. The SFP values correlated best with number of particles exceeding 20 μ (the pore size of the screen) and total volume of debris (Table 1).

Microaggregates in stored blood are stable with dilution and, consequently, attempts to correlate SFP and quantitative measurements of debris with the electronic counting method have been highly successful (6). Attempts at extending this correlation to microaggregates formed in whole blood or platelet-rich plasma (PRP) in response to an aggregating agent (ADP, epinephrine, or collagen) and to *in vivo* microaggregate formation in patients with shock and/or trauma were less successful. The SFP showed some variation in determining induced or *in vivo* aggregates but was usually elevated, whereas dilution for purposes of electronic particle counting consistently resulted in deaggregation.

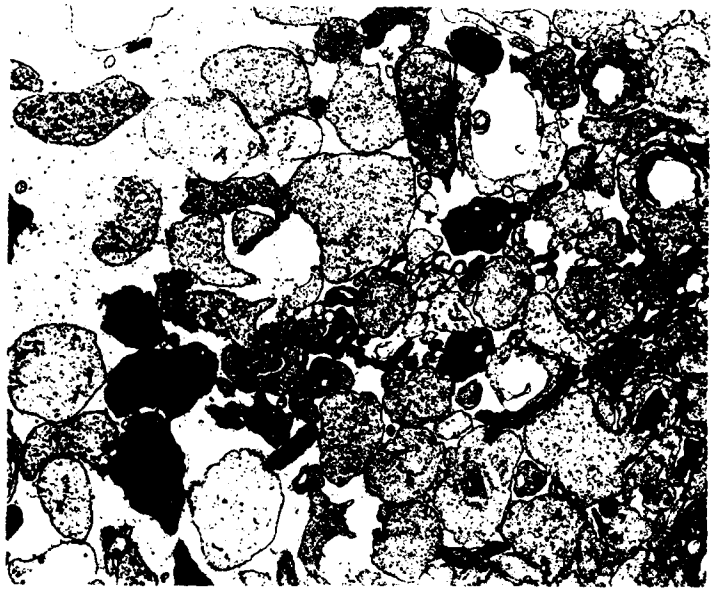

Fig. 1. Platelet microaggregates seen on smears of whole blood and platelet-rich plasma from stored blood

TABLE 1. CORRELATION OF SFP TO DEBRIS VOLUME AND POPULATION

COMPARISON OF SFP TO:		SAMPLE POPULATION n	CORRELATION COEFFICIENT r
Whole Blood	12.7-80.6 μ	16	.929
Debris Volume	20.2-80.6 μ	17	.646
Whole Blood	12.7-80.6 μ	17	.565
Debris Population	20.2-80.6 μ	17	.808

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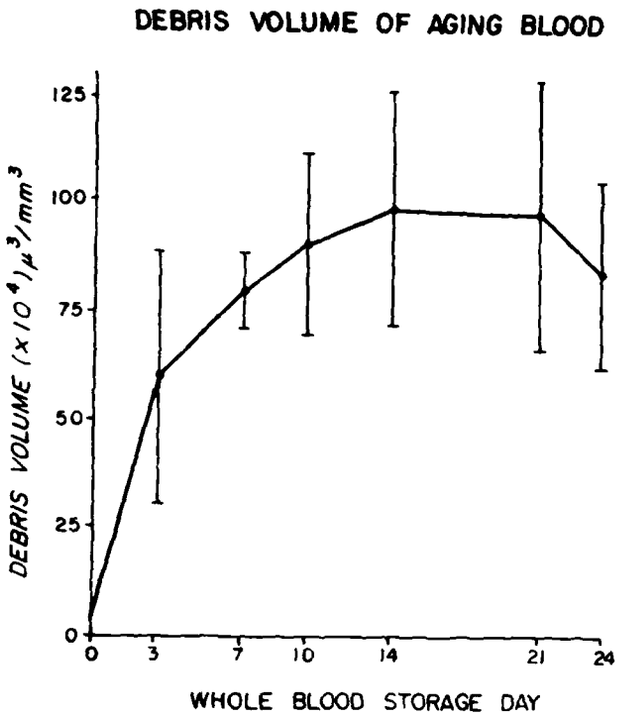

Fig. 2a. DEVELOPMET OF DEBRIS DURING STORAGE

Debris Volume of Aging Blood
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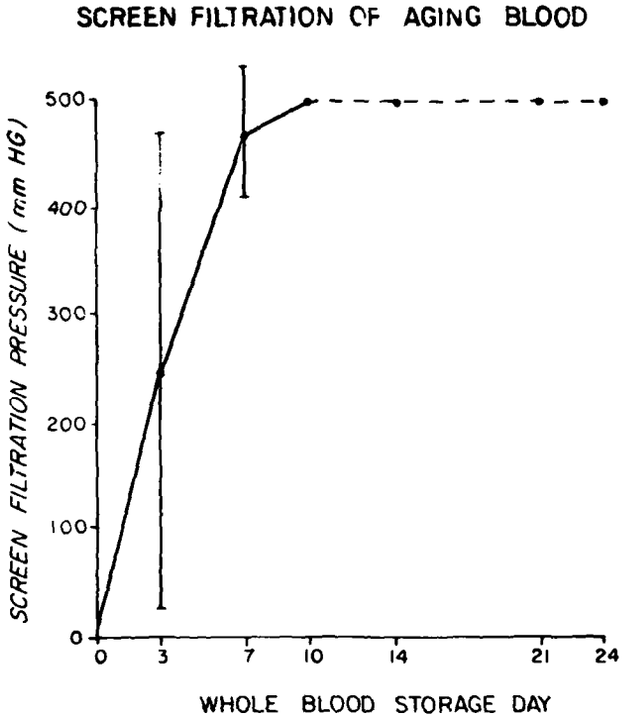

Fig. 2b. DEVELOPMENT OF DEBRIS DURING STORAGE

Screen Filtration of Aging Blood
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Clinical Significance of SFP.

The SFP has been used to study microaggregate formation in several clinical situations:

1) The usefulness of SFP in identifying and characterizing microaggregates in stored blood has been discussed above. We have previously used the technique to investigate the fate of stored blood microaggregates during transfusions in combat casualties. Although our data suggested that the material was removed by the pulmonary capillary bed, no direct evidence of any pathological consequences was obtained (7). Furthermore, data on microaggregate formation in response to trauma and shock suggest that some of our SFP increase may have been related to in vivo microaggregate formation at least in some patients (8, 9).

Numerous studies have attempted to establish evidence of pulmonary damage and microembolism as a consequence of microaggregates in stored bloods. Jenevein and Weiss (10) first called attention to the phenomenon of pulmonary platelet microemboli in patients having open heart surgery. Mosely and Doty (11) reported massive pulmonary microemboli in patients dying soon after battle injury. Hissen and Swank (12) and McNamara and co-workers (13) observed pulmonary hypertension in dogs infused with stored blood which was prevented by blood filtration. Litwin's group has reported changes in pulmonary function (14) and pulmonary structure and function (15) in dogs transfused with massive amounts of stored blood which were prevented by Dacron wool filtration. Similarly, Connell and Swank (16) have recognized pulmonary microembolism after blood transfusion using electron microscopy. Berman et al. (17) have noted increased capillary permeability in rats following stored blood transfusion and van Zandwijk (18), in exhaustive studies on isolated rabbit perfusions, has demonstrated pulmonary injury which was elicited by stored blood. Studies reported by Reul et al. (19, 20) in injured, masively tranfused patients have demonstrated a decrease in serious pulmonary insufficiency following fine screen filtration of transfused blood. All of the above evidence has led James (21), in a recent review of the significance of microaggregates in blood, to conclude that "it is likely that this (Reul's studies) will remain the only clinical trials because of the weight of laboratory evidence in the matter and the plain, good common sense of the matter."

Yet, the situation, in fact, remains unclear. First, no investigator to date has produced progressive pulmonary insufficiency with any combination of shock, trauma, and/or stored blood infusion in any animal species. Secondly, a number of studies including our own, have failed to demonstrate any significant abnormality in pulmonary structure or function in

baboons subjected to massive transfusion (22, 23) with or without shock and with or without trauma (24, 25). Bennett et al. (26) demonstrated changes in pulmonary structure and function, most notable in dogs but also in baboons, which were not influenced by microaggregate removal. Furthermore, clinical studies have failed to demonstrate that massive transfusion is a significant clinical correlate of patients developing posttraumatic pulmonary insuffiency (27, 28). Finally, the lesion with which we were concerned, that is, the syndrome of posttraumatic pulmonary insufficiency appearing on day 2 or 3 and peaking at a week is no longer a major problem in clinical care of postsurgery patients. That phase is nearly always successfully treated with innovations in respiratory care including PEEP, C-PAP, FIO2<50%, low pressure tracheostomy cuffs and volume-cycled ventilation. The common denominator to the adult respiratory distress syndrome (ARDS) which is the major clinical problem at present, develops the pulmonary problem beyond a week after the onset of severe systemic sepsis (29-31). The importance of blood microaggregates in the genesis of this phase of ARDS is then seriously in question. The evidence supporting the existence of microaggregates in stored blood is overwhelming but its pathological significance, if any, following transfusion remains an open question.

2) In vivo microaggregate formation as evidenced by increased SFP has been shown to occur in an experimental setting after shock in both dogs and baboons and in the dog after release of aortic ligation (9). Increases in SFP and concomitant decrease in microvascular perfusion have been reported in several experimental settings (8, 9, 21). Yet, studies by others have failed to demonstrate persistent organ dysfunction or evidence of capillary obstruction during the acute phase successful resuscitation from hemorrhagic shock (32, 33), although the finding of a high SFP acutely during shock is frequently observed experimentally.

It has, furthermore, been difficult to identify microaggregates in blood during hemorrhagic shock even when SFP is high. It now seems clear that microaggregates formed acutely in response to hypovolemic shock are unstable and deaggregate quickly with dilution of the study sample in vitro or resuscitation in vivo (34). Recent studies with tagged platelets demonstrating accumulation of the tags in visceral organ capillary beds suggest that microaggregates are formed and at least transiently trapped in visceral organs (32, 35). Whether they contribute to organ ischemia or are merely a consequence of the same has not been determined.

It is clear, however, that SFP is the best way to demonstrate *in vivo* aggregation until a technique for stabilizing *in vivo* microaggregates is established. It is of interest that the apparent deaggregation of *in vivo* microaggregates after dilution is identical to that known to occur to microaggregates formed *in vitro* by the addition of ADP, epinephrine, or collagen (6).

3) The SFP has been useful in identifying microaggregates induced by the cardiopulmonary bypass circuit during open heart surgery (36). Some of the recent evidence relating to microembolization employed a particle counting technique using a Doppler (37). It was not clear what the composition of the particles were, but subsequent studies using SFP and electronic particle counters suggest that few microaggregates are induced by the oxygenator or pump but rather most originate from the cardiotomy return (38-40). The use of a Dacron wool filter has virtually eliminated (40-41), therefore, the major sources of microemboli originating from blood; yet Doppler counts remain high (42) and it has been assumed that the majority of the remaining particles are microbubbles, the pathological significance of which remains undetermined. However, it is equally possible that aggregates originating from the cardiotomy line are relatively stable, having been induced by a variety of factors including tissue trauma and mechanical blood trauma from the suction system. On the other hand, those introduced by the oxygenator have just formed and may thus be readily reversible, similar to those seen with ADP-induced aggregates and shock. It is then important to compare SFP measurements and electron particle counting measurements in the oxygenator outflow in order to adequately assess oxygenator microaggregate formation. Should a technique be developed for fixing reversible aggregates it should be applied to the study of postoxygenator arterial blood.

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THE ORIGIN AND COMPOSITION OF MICROAGGREGATES FORMED IN THE STORAGE OF WHOLE BLOOD AND UNDER BLOOD BANK CONDITIONS

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Storage of whole blood for transfusion induces the formation of microaggregates, which are cause for concern because of their ability to pass through standard donor blood filters and enter the pulmonary circulation of the recipient (1-5). Jenevein and Weiss (6) identified emboli in small pulmonary vessels of transfused patients and showed these emboli to be the same, histochemically, as aggregates seen in donor blood. Belyakov, Kartashevsky and Rumyantsev (7) believed that aggregates, which they called microclots, produce microemboli and vascular spasm in the recipient. Swank (8,9) has shown an elevation of pulmonary artery pressure and an increase in alveolar dead space following the transfusion of blood rich in microaggregates.

Earlier studies by light microscopy have indicated that these aggregates consist of nuclear, cellular or amorphous debris, leukocytes, platelets, erythrocytes and fibrin (1,2,10-14). Solis and Gibbs (2,3), in determining the size and number of microaggregates with a particle size analyzer, and Swank (1), in measuring screen filtration pressure (SFP), have indicated that platelets and granulocytes are the major contributors to the formation of cellular aggregates. In this study, electron microscopy has been utilized to determine the composition of these aggregates, the time and frequency of their appearance, and their evolution in the course of storage of whole blood.

MATERIALS AND METHODS

Collection of Blood and Preparation of Buffy Coat.

The blood of healthy human donors was collected in Fenwell plastic bags containing acid citrate dextrose (ACD), formula A, using normal blood bank procedures. All blood was kept for 60-90 min at room temperature before proceeding with further preparations. Since the type of blood container, the frequency

of sampling and methods of blood suspension and centrifugation (14-16) could influence aggregate formation and composition, the treatment of blood after collection was varied in three ways:

Group 1: Blood was stored in the plastic collection bag at 4-6° C and aliquots were withdrawn sequentially for study during a 21-day period.

Group 2: Blood was stored as for Group 1 but only 1 aliquot was removed from each bag at the end of week 1 or 2 of storage.

Group 3: Blood was transferred to and stored in sterile plastic test tubes. This blood was not disturbed in any way; the buffy coat was allowed to form by spontaneous settling and was sampled at the end of week 1 and 2 of storage.

For Groups 1 and 2: When a sample of blood was desired for study, the Fenwall bags were inverted 15 times in a 30-sec-time period. In order to obtain a buffy coat rich in both platelets and leukocytes, aliquots of blood were transferred to plastic or siliconized test tubes and centrifuged at 5000-7000 g for 6-8 min. The platelet-poor plasma was pipetted off gently and the buffy coat was overlaid with 2.5% glutaraldehyde solution in 0.1 M phosphate buffer at pH 7.2. Since temperature may effect platelet aggregation (17), some of the above aliquots were maintained at room temperature (25° C) or at 4-6° C until fixation was completed. To eliminate the possibility that platelet aggregation might have been induced by centrifugation, some aliquots of blood were fixed in 0.1% glutaraldehyde, as described above, prior to centrifugation, as well as in 2.5% glutaraldehyde after centrifugation. The buffy coat which formed spontaneously in Group 3 blood was fixed as described above without centrifugation.

Techniques for Light and Electron Microscopy.

With each group of bloods, the buffy coat was fixed as described for 2 hr and then removed in its entirety from the test tube, cut in strips and washed in the 0.1 M phosphate buffer at pH 7.2. The strips were postfixed in 1% osmium tetroxide in phosphate buffer for 1 hr, then dehydrated in graded series of ethanol and embedded in Epon 812. For light microscopy (LM), sections were cut from Epon-embedded material at 1-2 μ, stained with toluidine blue or methylene blue-azure II. For electron microscopy (EM), ultra thin sections were stained with uranyl acetate followed by lead monoxide and examined with an HU-11C electron microscope.

RESULTS

Fresh Blood.

With LM, the buffy coat showed distinct free platelets in the upper layer and unaltered individual leukocytes in the lower layer. With EM, all platelets, whatever the mode of preparation, showed pseudopods, abundant glycogen, random distribution of intact granules and a narrow surface connecting system (Fig. 1). The contents of the latter were characterized by their amorphous appearance, identical to that of plasma protein, and by the absence, with few exceptions, of other components. Platelets fixed at room temperature were lentiform with a well-formed microtubular system, while cold fixation caused rounding of platelets and loss of microtubules. The latter observation is in agreement with the findings of Zucker et al. (18).

Stored Blood.

The cellular aggregates which developed during blood storage were classified in this study into three types according to their composition: Simple platelet aggregates (SPA), complex platelet aggregates (CPA) and neutrophil-platelet aggregates (NPA).

By LM, the first type of aggregate, SPA, began to appear as platelet clusters at 24 hr of storage. By EM, SPA were composed of a small number of rounded platelets which approximated each other over a limited surface area (Fig. 2). Platelet membranes were intact, although occasionally they merged with those of adjoining platelets. With storage, platelet granules underwent gradual and progressive changes. By day 1 of storage, granules, often centrally located, released their contents into the cytoplasm or the surface-connecting system. In the cytoplasm, the release material appeared as a round granular mass resembling a "bull's-eye" many times larger than the original granules and staining less intensely (Fig. 2). The bull's-eyes were never membrane bound but occasionally showed arrays of filamentous material at their periphery (Fig. 2). The contents of granules released into the surface connecting system assumed a variety of appearances (Fig. 2). On occasion, granules and cytoplasmic contents were seen in transit from inside the platelet into the surface-connecting system. The progressive decrease in the number of granules in aggregated platelets, as storage time was prolonged, indicated that the discharge of granules was a continuous process. By the end of week 3 of storage, few granules remained in the platelets. The size of SPA varied from 10 to 20 μ in diameter.

The second type of aggregates, CPA, first seen by LM on day 3 of storage, appeared to be larger than SPA. By EM, the aggregates were seen to be composed of densely packed platelets. In the peripheral portions of the CPA, platelets were large and saccular with sparse organelles (Figs. 3 and 4). Their plasma membranes either formed numerous pseudopodia (Figs. 3 and 4) or were obscured or absent (Fig. 5). The latter occurred more frequently as storage time was prolonged. Granule discharge in the CPA resembled that observed in SPA. In addition, with longer storage time, the bull's-eyes were found more frequently toward the centers of aggregates, the peripheral saccular platelets became depleted and the amount of platelet-free dense material in the central parts of the aggregates increased (Fig. 5).

The third type of aggregate, NPA, first seen by LM on day 3 or 4 of storage, was much larger than the SPA and CPA, i.e., as large as 200 μ. By EM, it consisted of individual or aggregated platelets, SPA and CPA, bound to extruded degenerated neutrophil nuclei. The sequence of events of neutrophil degeneration which culminated in the formation of these aggregates appeared to be as follows: 1) the perinuclear space of the neutrophil swelled (Fig. 6); 2) the nuclear and plasma membranes ruptured; and 3) the nuclear material escaping from the cell (Figs. 6 and 7) swelled, became ill-defined and faint staining by LM (Fig. 6) but the euchromatin-heterochromatin pattern remained identifiable by EM (Figs. 7 and 8). The extruding nuclear material flowed around the neutrophil (Fig. 7) and adhered to the surfaces of platelets, often engulfing them, thus forming NPA (Figs. 7 and 8). There were no intact neutrophils, other types of leukocytes, erythrocytes or fibrin in the NPA.

The evolution of free platelets into SPA, CPA and NPA is diagrammed in Figure 9. Once formed, all three types of aggregates could be recognized throughout the course of blood storage, regardless of the method of storage and preparation of specimens for examination (Table 1). The predominating aggregates at 21 days of storage were NPA and CPA with very few platelets remaining free or in SPA. The mode of storage and frequency of handling the blood influenced NPA formation. Blood which had been repeatedly sampled (Group 1) contained the greatest quantity, while blood which was handled only once (Group 2) had fewer NPA. Undisturbed blood (Group 3) had the fewest NPA despite the fact that it contained an abundant amount of degenerated neutrophil nuclei.

DISCUSSION

The present study has clearly defined the origin of the particulate material accumulating in stored blood. Sequential LM and EM observations made throughout the normal 21-day-storage period have provided evidence that only two blood elements - platelets and degenerated neutrophils - form this material and no other cellular or noncellular components of the peripheral blood such as fibrin, denatured protein, erthrocytes, intact neutrophils or other types of leukocytes participate.

Furthermore, three different types of aggregates have been identified and the evolution of one type into another has been recognized as well as the alteration of platelets and neutrophils responsible for this evolution. The cellular aggregates found early in stored blood are composed only of platelets clumped together first in a loose form, the SPA, and then in an irreversible form, the CPA. Within 3-4 days of storage, degenerating nuclear material from neutrophils is added to these aggregates cementing together free platelets, SPA and CPA thus forming still larger aggregates, the NPA.

The first two types of aggregates, SPA and CPA, have been previously reported to form sequentially in vitro (19-23) and in vivo (24-16) and are often termed loose reversible platelet aggregates and consolidated or contracted platelet aggregates. The loose approximation of platelets in SPA implies reversibility of this type of aggregate. However, the release of granule contents in SPA at 24 hr and the concurrent appearance of small focal points of membrane merger suggest that irreversible aggregation may have already begun at this time. In any event, within 3 days of blood collection, stable CPA have formed which could obstruct the pulmonary vasculature of the recipient.

A cause for greater alarm is the early appearance of the NPA which is not reversible and is remarkably larger than the SPA or CPA; this type of aggregate has not been previously described and, therefore, deserves close attention. Only the degenerated nuclear material from neutrophils interacts and adheres to platelet plasma membranes. This material does not penetrate or "lyse" the membranes since many platelets, although completely surrounded by nuclear material, appear absolutely intact, as can been seen in Figure 8. By contrast, platelet plasma membranes are readily destroyed by fusion with those of other platelets in the centers of CPA (Fig. 3) or by penetrating fibrin fibrils during coagulation (27).

Since the prerequisite for platelet-nuclei interaction is dependent upon both degeneration and extrusion of nuclear material from neutrophils, the question arises: what causes the

nucleus to extrude, swell and adhere? Extrusion is preceded by dilatation of the space between the double membrane of the nuclear envelope; hence, the dilatation may exert enough pressure upon the membrane to cause its rupture. Nuclear swelling which follows extrusion may represent a loosening of the very dense molecular packing of nucleoprotein in situ (28). Adherence of swollen, extruded nuclear material to the platelet plasma membrane could be due to an interaction between the negatively charged surfaces of platelets (29) and positively charged histones dissociated from nucleic acid. Whatever the physico-chemical character of the bond may be, it is unique and does not involve other available plasma membranes of intact leukocytes or erythrocytes. The appearance of neutrophils in the NPA brings to mind their role in arterial thrombosis where they occur more frequently than chance trapping would allow and are aligned onto the surface of platelet masses (30-32).

What causes platelets to aggregate during cold storage of blood? Exposure of platelets to cold without the intervention of thrombin promotes platelet aggregation (33). In addition, SPA and CPA could be mediated by thrombin concentrations too low to generate fibrin (34) or by adenosine diphosphate released from erythrocytes (35). Whatever the initial cause, there is a self-perpetuating mechanism for the progression of platelet aggregation in stored blood, viz, the release of platelet granules. In this study, the release appeared to be a continuous event leading to exhaustion. The release of contents of granules into stored blood may have an effect not only on stored donor platelets but also upon the platelets of the recipient of the blood. The cause of thrombocytopenia and hemostatic difficulties in massively transfused recipients could well be related to the extensive release of platelet procoagulant and adenosine diphosphate during storage.

Based upon findings presented here, a reconsideration of terminology is in order. In the past, a variety of names such as fibrin, clots, amorphous debris, nuclear or cellular debris and microaggregates of amorphous material were used (1,2,7,10-12,23,24) reflecting the uncertain origin and composition of the material which formed in stored blood. The word "clot" implies the presence of fibrin in the aggregates but this is absent. The term "amorphous debris" should be discarded since it reflects the limitations of light microscopy rather than composition. The term "nuclear debris" has a modicum of truth but is misleading because it neglects the invariable attachment of platelets to degenerated neutrophil nuclei. Since the platelets in CPA and NPA have undergone the release reaction, and are irreversibly adherent to each other, the terms "platelet thrombi" and "neutrophil-platelet thrombi" are commendable, especially

since platelet aggregates in the blood bag resemble *in vivo* arterial thrombosis. However, reservation of the term "thrombus" exclusively for *in vivo* phenomena speaks against the use of such a term for an *in vitro* storage event.

We, therefore, recommend the terms "Simple Platelet Aggregates", "Complex Platelet Aggregates" and "Neutrophil-Platelet Aggregates" because they specifically describe the cellular origin of these materials, connotate their origin *in vitro*, and imply their embolic character when infused into the vasculature of the recipient.

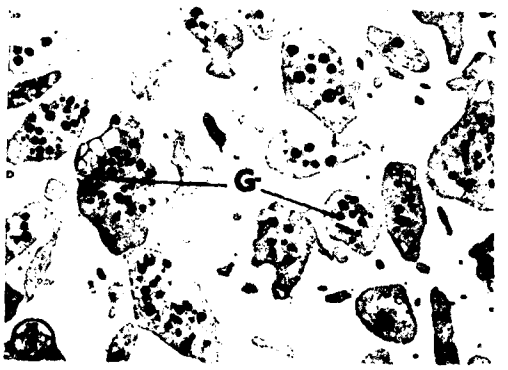

Fig. 1. The free platelets have intact granules (G) throughout the platelet layer. Cytoplasmic projections (pseudopods) are abundant. Day 0: X 3,600

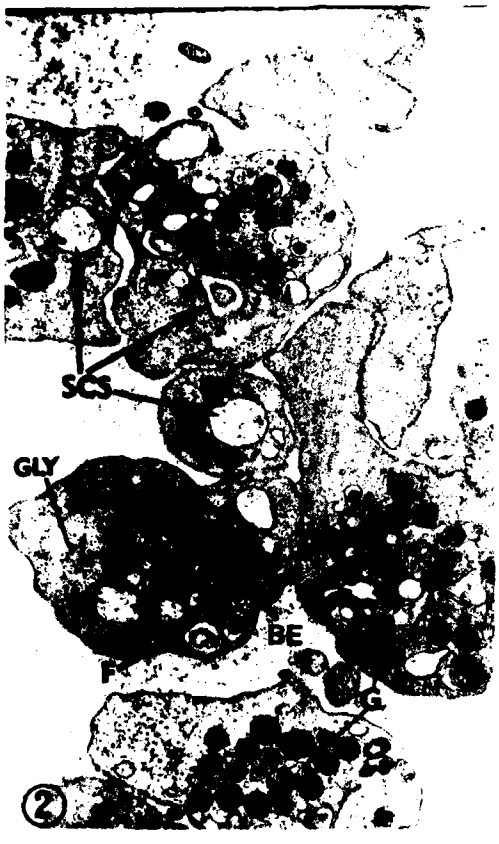

Fig. 2. Simple platelet aggregate. Surface membranes of platelets are approximated. Filaments (F) are seen around an early bull's-eye (BE) formation. The dilated surface-connecting system (SCS) contains a finely granular material, whose texture is identical to that of plasma, and a denser particulate material. (G) granules; (GLY) glycogen particles. Day 3: X 16,400.

Fig. 3. An early complex platelet aggregate. Platelets are crowded together and the surface membranes are extensively approximated or fused (square). In the periphery, platelets are saccular (S). Pseudopods are closely intertwined in the center (square). As in simple platelet aggregates, the surface-connecting system (SCS) is dilated. *Day 4: X 10,000.*

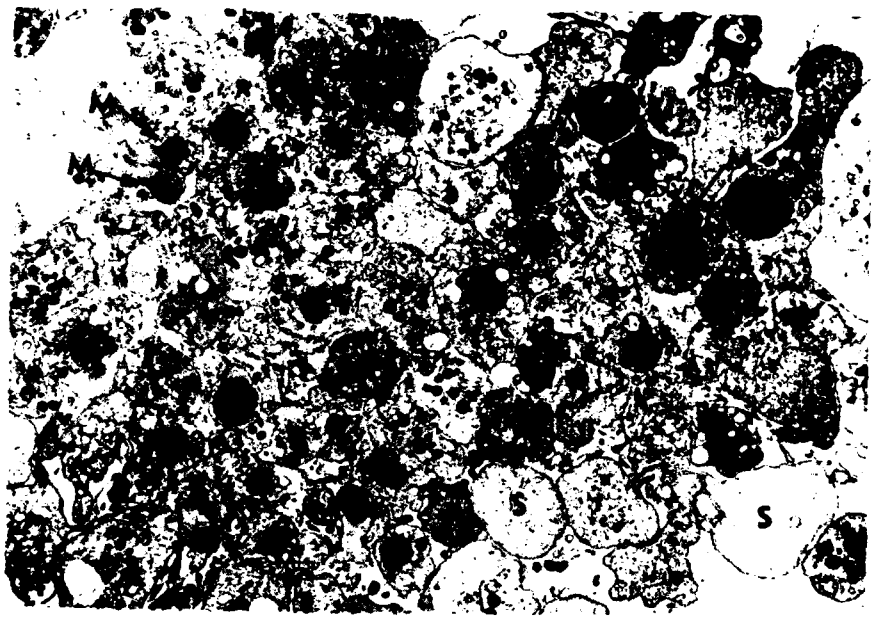

Fig. 4. This is a larger and more developed complex platelet aggregate than that seen in Figure 3. Saccular platelets (S) are seen in the periphery. Platelets (M) demonstrate a migration of the bull's-eye (arrows) to the center of the aggregate. Day 12: X 4,000.

Fig. 5. This is a late complex platelet aggregate. Platelets have disintegrated extensively in the center of the aggregate. Many platelets appear to have no granules while others have many crowded together (square). Peripheral saccular platelets (S), devoid of granules, pseudopodal formations and dilated surface-connecting systems are seen. Bull's-eye (BE). Day 12: X 8,000.

Fig. 6. Neutrophils are in the process of degeneration and show perinuclear swelling (small arrows). The degenerate nuclear material extruded from neutrophils (large arrows) stains less intensely than the nuclei of unaltered cells. Day 4: 1 μ Epon section stained with methylene blue-azure II. X 640.

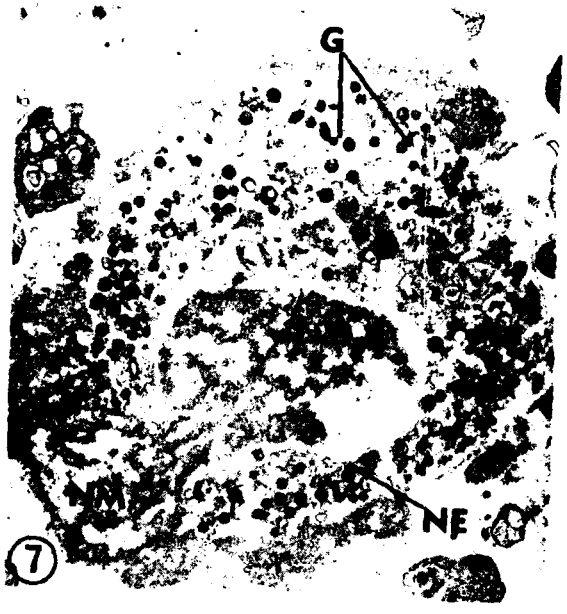

Fig. 7. Degenerating neutrophil, showing relatively well-preserved granules (G). Nuclear material (NM) which is in the process of extruding and spreading over the neutrophil surface has exited from the site where the double membrane of the nuclear envelope (NE) is ruptured. Day 4: X 10,000.

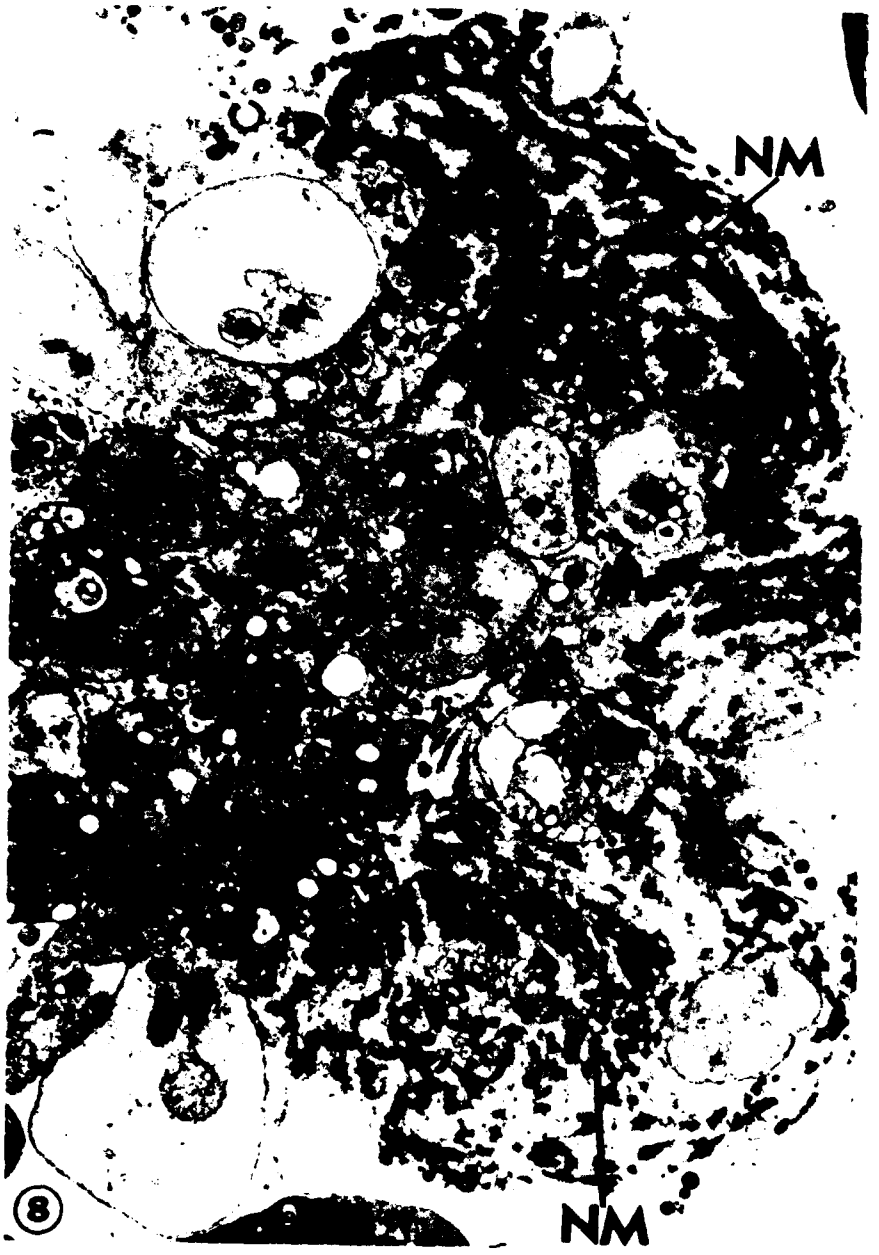

Fig. 8. Neutrophil-platelet aggregate. Nuclear material (NM) extruded from two degenerating neutrophils has adhered to a complex platelet aggregate which is centrally located. Day 6: X 14,000.

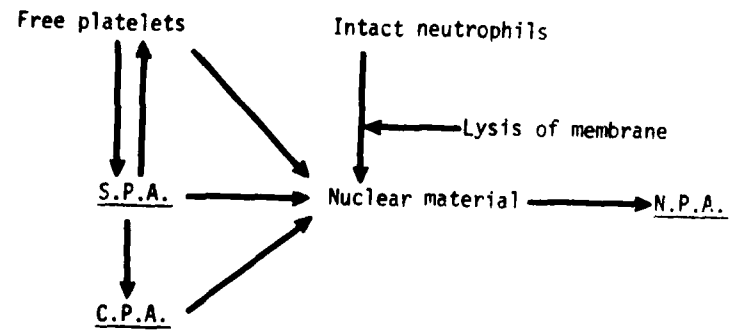

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Fig. 9. Schematic representation of the formation of the three types of aggregates. Free platelets give rise to simple platelet aggregates (SPA) which in turn progress to complex platelet aggregates (CPA) or could be dissociated into free platelets. Nuclear material derived from neutrophils can form neutrophil-platelet aggregates (NPA) upon contact with free or aggregated platelets.

TABLE 1. Time and Storage Mode in Relation to Aggregate Type and Total % of Platelets Aggregated.

Days of storage	Mode of storage	Simple platelet aggregate (SPA)	Complex platelet aggregate (CPA)	Neutrophil-platelet aggregate (NPA)	% of Platelet in aggregate (estimation)
0	group 1	0	0	0	0
1	"	++	0	0	10
3-4	"	++	++	+	20
	"	++	+++	++	60
6	group 2	++	+++	+	60
	group 3	++	+++	+	50
11-12	group 1	++	+++	++++	80
	group 3	++	+++	++	60
14-15	group 1	++	+++	++++	90
	group 2	++	++++	++	90
17-19	group 1	++	++	++++	90
21	group 1	++	++	++++	90

Code: 0 = not present; + = rare; ++ = present, but not abundant; +++ = abundant; ++++ = predominant type of aggregate, far more than all others combined. % platelets in aggregate is the percent of the total platelet population that has entered any one of the 3 different types of aggregates.

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PLATELET THROMBOEMBOLISM IN HUMAN DISEASE

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The role of platelets in the pathogenesis of thromboembolic disease has consumed the investigative energies of many clinical and basic science researchers during the past decade. The resultant discoveries have advanced the knowledge of the pathophysiology of thrombosis and disorders of hemostasis greater than any other area of endeavor. The purpose of this review is threefold: 1) to assess mechanisms by which platelets might produce organ damage, 2) to evaluate disorders in which platelet function contributes to the pathogenesis, and 3) to consider the pharmacology of platelet function inhibiting drugs and their clinical usefulness in preventing and treating these disorders.

ORGAN DAMAGE BY PLATELETS

Organ damage and dysfunction may be an important consequence of platelet aggregation and lodging of platelet microemboli in the microcirculation (108). The most obvious mechanism by which platelets might produce damage is by mechanical obstruction of vascular beds. Platelets circulate in a discoid shape with a diameter of approximately 2 μm and occupy a volume of about 7 μ^3. However, under the influence of several aggregating agents they assume a spherical shape and an increase in volume (144). Even so, the maximum possible total volume occupied by all of the circulating platelets in a normal adult is only about 10 ml. Obviously, the obstructive potential of pure platelet thromboemboli in medium- and large-sized vessels is limited. In the microcirculation, however, occlusive platelet clumps in critical regions might result in considerable morbidity and even death. Such a mechanism is supported by several experimental observations. Jorgensen et al. (83) noted that intermittant infusions of adenosine diphosphate (ADP) into the coronary circulation of pigs were associated with circulatory collapse, ECG evidence of myocardial ischemia, ventriculat dysrhythmias, and formation of platelet aggregates in the microcirculation. Similar experiments carried out in rabbit kidneys resulted in renal ischemia, acute and chronic hypertension, presumably by renin release, and nephrosclerosis (85,107). In another study (153), platelet thrombi in the microvasculature of the lung caused by infusion of sodium arachidonate resulted in immediate death of the animal. These lethal effects could be prevented by prior treatment with aspirin. Autopsy studies demonstrating platelet-rich emboli in the distal coronary tree of patients dying suddenly of myocardial ischemia (138) and in the pulmonary microvasculature of some individuals dying with a clinical picture of acute respiratory

failure (129) attest to the significance of this mechanism.

The obstructive potential of platelet aggregates is considerably enhanced by the interaction with the coagulation system. Blood coagulation is accelerated in the presence of platelets. This property is referred to as platelet factor 3 and is felt to reside in the phospholipids and lipoproteins present in platelet membranes (95). Platelet factor 3 activity is not present in unstimulated platelets but is made available during platelet aggregation. Blood coagulation is further amplified by platelet coagulant activities, felt to be distinct from platelet factor 3, which appear to be able to initiate and accelerate intrinsic coagulation (15,173). Again, these properties are felt to be engendered by platelet aggregation. The resultant fibrin formation and entrapment of cellular elements from the blood would serve to greatly increase the mass of platelet aggregates.

Platelet aggregates may also cause direct injury to the vessel wall. Hughes and Tonks (80) initially called attention to the lung and heart lesions resembling vasculitis which were associated with intravascular platelet clumping in rabbits. Other investigators (84) have noted extensive vascular injury in swine and rabbits produced by platelet aggregation from infusions of ADP. Varying degrees of endothelial injury have been noted beneath platelet aggregates in animal preparations (7) and has been thought, by some (86), to be secondary to the process of platelet aggregation. Lough and Moore (93) demonstrated that this might be due to elaboration of thrombin during aggregation.

Perhaps one of the most important platelet functions potentiating organ dysfunction and damage is the "platelet release" reaction. Platelet release, which occurs during platelet aggregation, is initiated by a variety of chemical and physical stimuli including thrombin, collagen, ADP, serotonin, epinephrine, gamma globulin, endotoxin, antigen-antibody complexes, emulsions of long-chained saturated fatty acids, and prosthetic surfaces (79,117). Substances released form the $\underline{\alpha}$-granules and dense bodies of platelets into the surrounding medium include adenine nucleotides (ADP, ATP), serotonin, epinephrine, histamine, and other biologically active compounds (117). These substances may promote vasoconstriction or vasodilatation resulting in shifts of blood flow away from critical areas. Such a mechanism has been postulated for the rise in pulmonary vascular resistance and decrease in lung compliance in experimental preparations where platelet release is induced (169). In one study (183), increased pulmonary vascular resistance, decreased compliance, and increased physiologic shunt were correlated with prolonged transit of radio-labelled platelets through the pulmonary circulation.

These findings led the authors to postulate a platelet-endothelial/subendothelial interaction from capillary damage resulting in local release of biologically active amines. In other studies carried out by Zervas et al. (192,193) in dogs, cerebral arterial vasospasm which retularly occurs when autologous or homologous blood is injected into the cisterna magna was inhibited by depleting the platelets of serotonin and catecholamines by treatment with reserpine.

Recent advances in research in platelet prostaglandin metabolism have identified other substances which might be responsible for modulating vascular reactivity. Cyclic endoperoxides (prostaglandins G_2 and H_2) can be generated from arachidonic acid via a cyclo-oxygenase system present in platelets (68). The labile cyclic endoperoxides are rapidly converted to even more unstable compounds termed thromboxanes A_2 and B_2 (69). The cyclic endoperoxides and thromboxanes are produced during aggregation of platelets and themselves are capable of producing platelet release and aggregation. In addition, thromboxane A_2 produces potent contractions of arterial smooth muscle in vitro (69), and the generation of this substance from platelets has been postulated to be responsible for coronary artery vasoconstriction in some cases of myocardial ischemia (46). Prostaglandins G_2 and H_2 from platelets can also be converted to intermediates which produce vasodilatation. Moncada et al. (105,106) have identified a substance termed prostaglandin X or prostacyclin, a compound that inhibits platelet aggregation and produces vasodilation, which is generated when platelets are incubated with microsomes from arterial and venous walls. Conceivably, then, platelets could produce vasoconstriction or dilation under different circumstances resulting in shifts of blood flow, ischemia, and organ dysfunction.

Another adverse consequence of the platelet release reaction is an increase in vascular permeability leading to edema. Single platelets are important in maintaining vascular integrity (9,89). However, following platelet aggregation, vascular permeability factors are released resulting in a local increase in vascular permeability. Mustard et al. (113) originally called attention to this phenomenon and suggested histamine, serotonin, and lysosomal enzymes from platelets as possible agents. Nachman et al. (120) have characterized a permeability factor in rabbit platelets that is a heat stable, nondialyzable cationic protein fraction with a molecular weight of approximately 30,000. Experimentally, Vaage et al. (168) noted that collagen-induced platelet aggregation caused an increase in the net filtration rate of in vitro perfused-rabbit lungs, presumably from an increase in vascular permeability. The relevance of these experimental observations to human disease is, at present, unknown.

The above mechanisms have dealt primarily with acute changes in organ function stemming from platelet activities. Chronic changes may be brought about by platelets participating in the development of arteriosclerosis. A platelet mitogenic factor has been identified which stimulates the growth of vascular smooth muscle (142). Proliferation of smooth muscle cells beneath areas of endothelial injury represent preatheroslerotic lesions, the development of which are inhibited by thrombocytopenia (109) or treatment with dipyridamole (73) in experimental animals. The contribution of platelet function to the pathogenesis of arteriosclerosis is receiving increasing attention and the subject has been recently reviewed (118,143).

THROMBOEMBOLIC DISEASE

Turning now to clinical disorders associated with platelet aggregation and embolization, it is helpful to suggest criteria ascertaining the importance of this pathogenetic mechanism. Of first concern is that platelet aggregates or platelet-rich thromboemboli should be demonstrable in biosy and autopsy materials from patients suffering from the disease in question. Obviously, the deranged organ producing clinical manifestations should contain the platelet aggregates. Next, supporting evidence may be available from abnormalities in _in vitro_ and _in vivo_ platelet function tests. For example, enhanced platelet reactivity as measured by platelet aggregometry, platelet adhesiveness, and other _in vitro_ tests, and increased platelet consumption as measured by shortened platelet survival time are clearly associated with many of the disorders under consideration. Lastly, pharmacologic inhibition of platelet function should forestall, prevent, or ameliorate the disorder in question.

The state of the art is such that one cannot be too rigid and these criteria should be viewed as loose guidelines. For instance, the evanescence of some platelet aggregates makes histological demonstration difficult. Further, abnormalities in _in vitro_ platelet function tests suggesting enhanced platelet reactivity are not found by all investigators. It is also unknown whether such _in vitro_ findings truly reflect a predisposition to thromboembolic disease. Finally, the inhibition of platelet function offered by drugs currently in use may be inadequate. Data from negative clinical trials, therefore, render interpretation of the pathogenetic role of platelet function in a particular disease difficult.

Transient Ischemic Attacks.

Early studies of transient cerebral ischemic attacks suggested that the source of embolic debris was thrombotic

material forming on atherosclerotic lesions in the extracranial arteries leading to the brain (103). These findings provided the rationale for chronic anticoagulant therapy in reducing the frequency of attacks and, ultimately, for preventing stroke (26,103,182). More precise identification of the embolic material awaited the elegant fundoscopic observations of Fisher (53) who observed "pale, intravascular substance" or white bodies moving through the retinal arteries during an episode of amaurosis fugax. Later histological studies determined that this substance was composed predominantly of platelets (97).

The role of platelets in the pathogenesis of transient cerebral ischemia has been strengthened by the many studies demonstrating increased platelet reactivity in this disorder (102). Enhanced sensitivity to aggregating agents (35,70,188), increased retention of platelets in glass-bead columns (187), greater numbers of circulating platelet aggregates (41,184), high levels of platelet coagulant activities (175) and shortened platelet survival (1,72) have been associated with transient cerebral ischemia. However, since the diagnosis was established before platelet function testing was performed, it is not clear whether these abnormal tests reflect a predisposition to attacks or, rather, that the enhanced platelet function is secondary to the attacks. Even if the latter is the case, enhanced platelet reactivity might increase the severity of a single attack and increase the likelihood of subsequent attacks (41).

These considerations have led to the use of antiplatelet drugs in this disorder. Long-term anticoagulant therapy has been evaluated and has been shown to reduce the frequency of attacks and decrease the risk of stroke (182). However, the difficulties in management and complications, expecially in cerebral hemorrhage (26), have made this form of therapy unpopular. The ease and safety of antiplatelet agents is attractive. This is especially so in patients unable to undergo carotid endarterectomy or other vascular reconstructive procedures.

The ability of antiplatelet agents to reduce the frequency of transient cerebral ischemic attacks is suggested by several recent studies. Retrospective studies and anecdotal case reports support aspirin as an effective agent (43,75). In a double-blind crossover trial conducted by Evans (52), sulfinpyrazone significantly reduced the frequency of attacks in comparison to a placebo. More definitive, large-scale trials in the U.S. (evaluating aspirin versus placebo) and Canada (evaluating sulfinpyrazone versus aspirin versus both drugs given together) are in progress (172). In contrast to the promise offered by aspirin and sulfinpyrazone, dipyridamole has been reported ineffective in reducing the frequency of attacks

in a controlled, double-blind study(2). In another trial by the same investigators (3), clofibrate was also found ineffective.

Prosthetic Devices in Contact with Blood.

Platelets rapidly accumulate on nonendothelialized surfaces in the arterial circulation (147). Thromboembolism complicating prosthetic heart valves (71,177), artifical vascular conduits (51), arteriovenous cannulae used for hemodialysis (88), vascular catheters, oxygenators for extracorporeal bypass (155), and dialysis membranes (92) is a major cause of morbidity associated with the use of such devices (14). Further, build-up of platelet-rich thrombotic deposits can result in dysfunction of the device. Clinical manifestations in patients with substitute heart valves are produced by large vessel occlusion resulting in stroke, blindness, and peripheral or visceral ischemia and tissue necrosis. Other prosthetic devices such as oxygenators used in extracorporeal bypass circuits produce myriads of platelet microemboli (155) which may produce subtle clinical manifestations such as postoperative cerebral dysfunction (19). Hill and colleagues (77) have demonstrated a high incidence of emboli in the cerebral circulation of patients dying after open-heart surgery.

Platelet survival time is shortened in many patients with prosthetic heart valves (71,159,161,176,177) and is inversely correlated with the surface area of the valve and the incidence of thromboembolism (71). Selective platelet consumption is further documented by normal fibrinogen survival in these patients. Homograft valves do not significantly shorten platelet survival and have a low incidence of thromboembolism (159,177). All of these studies support the thesis that platelet adherence and aggregation is occurring at the prosthetic valve surface with thromboembolism as a consequence. In these patients, the platelet survival time is the most sensitive measure of these events since *in vitro* platelet function tests such as aggregometry and retention in glass-bead columns are normal (177). Weily and Genton (176) demonstrated that antiplatelet therapy with sulfinpyrazone normalized the shortened platelet survival in patients with prosthetic heart valves. At the same time, Harker and Slichter (71,72) found that dipyridamole produced the same result. Interestingly, this study and a subsequent one failed to document an influence of aspirin on platelet survival, although aspirin appeared to potentiate the effect of dipyridamole. The clinical relevance of these studies is borne out by the reports of Sullivan *et al.* (162,163) showing that dipyridamole administered along with warfarin reduced the frequency of prosthetic valve embolism when compared with warfarin alone.

Myocardial Infarction.

Roberts and Buja (138) in a necropsy study of patients dying from acute myocardial infarction questioned the significance of macroscopic coronary arterial thrombi in this disease. Coronary thrombotic occlusion was found in 54% of patients with transmural necrosis, 8% of patients who died suddenly, and none in those with subendocardial necrosis. These findings and those of others (156) suggested that coronary artery thrombi were the consequence rather than the cause of infarction in a substantial number of patients. They further raised the possibility that the pathogenesis of infarction in such patients was obstruction of the coronary microcirculation by platelet microemboli. These findings were substantiated by Erhardt et al. (49) who studied isotope distribution in coronary thrombi from autopsied patients who had received ^{125}I fibrinogen on admission to the coronary care unit. The majority of thrombi were diffusely radioactive suggesting formation after infarction.

Experimentally, platelet aggregation and microembolization induced in the coronary microcirculation produce acute myocardial ischemia, dysrhythmias, circulatory collapse and patchy myocardial necrosis without causing main coronary artery occlusion (66,67,83,116). This mechanism has been suggested as an alternative to large vessel thrombosis in many patients with myocardial infarction. Whether this is true remains controversial (28). Even so, platelets are probably important in the pathogenesis of myocardial infarction either by participation in the formation of occlusive thrombi or by microembolization of aggregates.

The results of platelet function testing in patients with coronary artery disease have been variable. Steele et al. (157) were unable to correlate severity of coronary artery disease with abnormalities in platelet function studies. For a period of days to weeks following infarction, most investigators find increased platelet sensitivity to aggregating agents (70,188), spontaneous platelet aggregation (185) and increased platelet retention in glass-bead columns (124,187). Further, platelet survival is shortened for 2-3 weeks following infarction but then returns to normal (1). These studies suggest that enhanced platelet reactivity is secondary to myocardial infarction. However, O'Brien et al. (125) studied patients long after (months to years) myocardial infarction and found abnormal platelet function. This led these investigators to postulate a "thrombotic diathesis" which predisposed some individuals to myocardial infarction. In support of this thesis, cigarette smoking, a strong risk factor for myocardial infarction, is associated with enhanced platelet aggregation, an effect possibly mediated through release of catecholamines (91).

Despite the unclear role of platelets in the pathogenesis of myocardial infarction, prevention by inhibition of platelet function is receiving increasing attention. It is hoped by preventing platelet participation in occlusive thrombi and inhibiting platelet microembolization that the mortality and morbidity of myocardial infarction will be reduced. Experimentally, Haft et al. (66) were able to prevent myocardial necrosis in dogs infused with catecholamines by pretreatment with platelet inhibitors. Clinically, epidemiologic studies conducted by Jick and associates of the Boston Collaborative Drug Surveillance Group (18) demonstrated a highly significant negative association between regular aspirin ingestion and myocardial infarction. Subsequently, Elwood et al. (47) in a randomized double-blind trial, compared aspirin versus placebo administered to males with recent myocardial infarction. Overall, they could not discern a statistically significant reduction in recurrent myocardial infarction in subjects taking aspirin, although there was a trend favoring this group. Also, the mortality for recurrent infarction was significantly less for the aspirin group among subjects entered into the trial less than 6 weeks after initial infarction. Clofibrate, which has platelet-suppressant activities, as well as an effect of lowering cholesterol levels, has been evaluated in three prospective, randomized, double-blind trials (34,65,134). In two, clofibrate-treated patients had significantly fewer mortalities from myocardial infarction (65, 134). In the other, generally felt to be a better designed and executed trial (59) clofibrate was without significant benefit (34). In another well-designed trial, Gent et al. (58) evaluated dipyridamole versus placebo administered to patients with recent myocardial infarction and could not discern a significant benefit of the drug in terms of reinfarction, overall mortality, or other thromboembolic events. Blakely and Gent (17) administered sulfinpyrazone to elderly institutionalized males and noted a significant reduction in mortality for all patients as well as an important subgroup who had prior myocardial infarction when compared to placebo-treated controls.

Although it is far from clear whether antiplatelet therapy can prevent myocardial infarction the attractiveness of such an approach has stimulated many clinical trials. At present, there are more than five large-scale multicenter trials in progress here and abroad (90). The drugs being evaluated include aspirin, dipyridamole, sulfinpyrazone, clofibrate, and dipyridamole combined with aspirin. Data from some of these trials may be available soon and should help to answer this important question.

Renal Disease and Allograft Rejection.

Studies of biopsy material have documented platelet aggregates in the glomeruli of kidneys in individuals with glomerular disorders (42) and renal transplant rejection (130). Claes (30), using ^{51}Cr-labelled platelets and ^{125}I-labelled fibrinogen, demonstrated the accululation of platelet radioactivity in rejecting dog kidneys before the accumulation of radioactive fibrin. Progressive accumulation of both correlated with decreases in renal blood flow (31). The accumulation of platelets far exceeded that of fibrin and correlated with the histological finding of platelet-rich thrombi in glomerular capillaries. This group of investigators (151), as well as others, could delay functional, isotopic and histologic manifestations of rejection by prior treatment with antiplatelet agents such as cyproheptadine, dextran, aspirin and sulfinpyrazone. From presensitization experiments, it is hypothesized that antibodies directed against the endothelium of the donor organ result in antigen-antibody complexes which lead to platelet aggregation (98,152).

In vitro platelet function testing in children suffering from acute and chronic glomerular disorders demonstrated enhanced aggregation in response to ADP and collagen (10). These findings correlated with the degree of proteinuria leading the authors to suggest that active glomerular disease resulted in loss of circulating proteins normally inhibitory towards platelet aggregation. George et al. (60) found shortened platelet survival and selective platelet consumption in patients with the hemolytic-uremic syndrome, membrano-proliferative glomerulonephritis, and rejecting renal allografts. They also found that treatment with combinations of dipyridamole, aspirin, heparin and warfarin had no effect on platelet survival or renal function. Immunosuppression in transplant patients, however, improved both of these parameters. This supports the concept that small-vessel endothelial damage from immune mechanisms is the primary event.

There have been few long-term studies evaluating antiplatelet therapy in these disorders. Trygstad et al. (167) conducted a controlled, prospective, double-blind study of aspirin in children with proliferative glomerulonephritis and the nephrotic syndrome. Despite suppression of platelet aggregation, they could detect no benefit of aspirin on the course of the disease. Mathew et al. (96) reported a controlled trial evaluating dipyridamole plus warfarin administered to patients with cadaver renal allografts. Although there was no beneficial effect on graft survival or renal function, there was a significant reduction in vascular and glomerular lesions appearing in renal biopsy specimens in the patients in the treatment group. More

clinical data are needed in this area.

Shock, Massive Transfusion, and Pulmonary Failure.

Histologic demonstration of platelet-rich microthrombi in the pulmonary vasculature of patients dying from pulmonary failure following shock, trauma, and massive blood transfusion led to the consideration of platelet aggregation as a contributory factor in this setting (16,44,81,112). Zapol and Inider (190) have recently documented dramatic increases in pulmonary vascular resistance in patients with severe respiratory failure. They postulate that diffuse pulmonary vascular obstruction partly from platelet-rich thromboemboli contribute to this picture (189). Their findings of shortened platelet survival in these patients support this contention (148).

In experimental settings, dogs subjected to hemorrhagic and endotoxic shock develop elevated screen filtration pressures (assumed to be dependent upon platelet function) in pulmonary arterial blood (94,164,166). The finding of platelet clumps in the lungs of dogs subjected to regional ischemia complemented these findings (64). These findings, however, have not been well-documented in humans and the relevance of these experiments is uncertain.

Stored blood contains particulate matter (termed "microaggregates") consisting of platelets, white cells, red cell fragments, and fibrin, the content of which increases with the length of storage (100,111,165). McNamara et al. (99) found abnormally high screen filtration pressures in the superior vena caval blood of combat casualties who were simultaneously receiving large amounts of banked blood that also had high screen filtration pressures. The fact that simultaneously drawn femoral arterial blood had normal screen filtration pressures suggested that the particulate matter was being trapped in the lung thus contributing to pulmonary failure.

Whether microaggregates can produce abnormalities in pulmonary function has been addressed in experiments where dogs were transfused with blood containing large amounts of particulate matter. One group of investigators using this model has reported pulmonary hypertension associated with an increase in pulmonary shunting of blood and a decrease in pulmonary diffusing capacity (11,39). Conflicting data were reported by Giordano et al (62) who could demonstrate no changes attributable to the infusion of large amounts of microaggregates.

The role of platelets in the formation of particulate matter in stored blood is uncertain since other cellular elements and debris are present. Furthermore, the role of microaggregates

in the pathophysiology of pulmonary failure following shock and massive transfusion is not clear. Despite the availability of a variety of effective filtering devices that remove debris from banked blood (61), convincing clinical data demonstrating clear benefit from the use of these filters is scarce (135). The expense entailed by the use of such devices must be justified by well-designed clinical trials proving their worth.

Venous Thromboembolism.

The ability of conventional anticoagulants to prevent formation of venous thrombi as well as inhibit the growth of established thrombi prove the importance of the coagulation system and fibrin formation in the pathogenesis of this disorder (33). However, the histological finding of platelet-rich, early thrombi behind venous valves and at areas of endothelial injury (56,128,150) suggests that platelets might have an important role in the initial stages of venous thrombosis.

Platelet-function testing in venous thrombosis has received attention. In idiopathic recurrent venous thrombosis, enhanced platelet aggregation, circulating platelet aggregates, spontaneous platelet aggregation, increased platelet retention in glass-bead columns and shortened platelet survival are found (78,158,186). These abnormalities are present between, as well as during, clinical attacks suggesting that abnormal platelet function is a causative factor in this form of venous thrombosis. In the far more common form of venous thrombosis that develops in the postoperative state, platelet-function testing is less discriminative. Walsh (174) found increased platelet coagulant activities in patients who developed venous thrombosis following total dip replacement. Because these abnormalities were not present preoperatively, however, it is unclear whether they were causative or, rather, nonspecific phenomena associated with thrombosis. Harker and Slichter (72) found shortened platelet survival in clinically overt postoperative venous thrombosis which they attributed to passive consumption of platelets in the thrombotic process. In other studies earlier venous thrombosis (detected by labelled fibrinogen scanning) was not discriminated by platelet survival which was already shortened due to the postoperative state (32).

Pharmacologic inhibition of platelet function offers insight into the role of platelets in the pathogenesis of venous thrombosis. In idiopathic recurrent venous thrombosis, treatment with sulfinpyrazone or a combination of aspirin and dipyridamole reduces the frequency of attacks and normalizes platelet survival (158,186). In postoperative venous thrombosis, the lines of evidence are less clear. Dextran has generally been found effective in preventing venous thrombosis in over 16

controlled trials (33). Dextran, however, in addition to its platelet inhibiting properties, adversely influences fibrin polymerization (119) and improves venous blood flow (149) and these effects may be more important in preventing venous thrombosis. Dipyridamole has been evaluated in a number of trials and has been found ineffective in preventing venous thrombosis (20,145). Hydroxycholoroquine sulfate has been found effective in three controlled trials (22,23,29). Aspirin, perhaps the most controversial drug of all in this regard, has been found equivalent to warfarin and dextran in protecting against venous thrombosis in orthopedic patients (74,145). In one report, aspirin significantly reduced the incidence of autopsy-proven fatal pulmonary embolism in patients with hip fractures (191). In other surgical patients (general, gynecological and urologic) the data on aspirin are conflicting. Some investigators report a significant protective effect (32) while others do not (133). In these patients, the usefulness of this drug as an antithrombotic agent is uncertain. Sulfinpyrazone has not been evaluated for the prevention of postoperative venous thrombosis.

POTENTIALLY USEFUL ANTIPLATELET DRUGS

A wide variety of drugs interfere with platelet function and a number of excellent reviews on the subject have recently appeared (40,171,180,181). This section will be concerned only with drugs which appear clinically promising in the management of thromboembolic disease. Several difficulties arise with interpretation of available data. Definition of antiplatelet properties of a drug depends, primarily, on its ability to produce abnormal results in *in vitro* and *in vivo* test systems. Logically, a drug which produces an *in vitro* effect such as inhibiting platelet release and aggregation should alter *in vivo* tests such as the bleeding time and platelet survival. However, such is not the case. For example, aspirin which has a strong *in vitro* effect, appears to have no influence on platelet survival shortened by prosthetic heart valves. Conversely, dipyridamole which possesses comparatively weak *in vitro* effects normalizes platelet survival in patients with prosthetic heart valves and appears to be clinical useful in preventing valve thromboembolism. Further, the relevance of *in vitro* and *in vivo* platelet-function testing to thrombogenesis is open to question. Precisely which platelet functions, as defined by *in vitro* and *in vivo* tests, are most pertinent to thrombogenesis in unknown. Thus, it is impossible to accurately predict the clinical utility of a drug based on platelet-function testing. As a corollary, one cannot say that one drug holds more promise than another simply because it has more of an effect upon certain tests. To compound matters, a drug beneficial in one thromboembolic disorder may be without effect in another. As an example,

dipyridamole, which appears beneficial in reducing thromboembolism from prosthetic heart valves, probably has no influence on transient cerebral ischemic attacks; sulfinpyrazone, however, appears effective in both disorders. The ultimate clinical utility of candidate antiplatelet drugs will be decided by well-designed clinical trials. Many are in progress and forthcoming data should clarify the problems.

With these caveats as an introduction, this section will discuss each drug in terms of current understanding of mechanism of action, effects on in vitro and in vivo platelet-function tests, and assessment of clinical utility based on published clinical trials.

Acetylsalicylic Acid (Aspirin).

The mechanism by which aspirin inhibits platelet function is probably based upon its ability to inhibit platelet prostaglandin synthesis. Labile endoperoxides (PGG_2 and PGH_2) which induce platelet aggregation are produced from arachidonic acid by platelet cyclooxygenase (68). This enzyme system is specifically inhibited by aspirin and other nonsteroidal, antiinflammatory drugs in that the antiplatelet effect persists beyond the time the drug is present in the circulation (123). Platelet function abnormalities are detectable 7 days after taking a single tablet (160). This may be due to acetylation of the platelet membrane by aspirin since this chemical alteration is permanent (140). Sodium salicylate, which lacks an acetyl group, has little or no effect on platelet function (122,179).

In vitro platelet-function tests are profoundly influenced by aspirin. Aspirin specifically blocks platelet release of ADP, serotonin, and other substances (50,122,179). In the presence of aspirin, platelets will participate in primary or first phase aggregation in response to added ADP, collagen, or epinephrine. However, because endogenous ADP is not released, secondary or second phase aggregation does not occur (194). Platelet retention in glass-bead columns is modestly depressed by aspirin (160) and platelet adhesion to subendothelium and collagen-coated surfaces has been reported to be defective (25). It is difficult to study platelet adhesion separate from aggregation, however, and others have reported that platelet adhesion to subendothelium is not inhibited by aspirin (12).

The bleeding time and platelet survival are important measures of in vivo platelet function. The bleeding time is regularly prolonged in normal individuals by aspirin ingestion (179). On the other hand, aspirin appears to have no effect on platelet survival shortened by prosthetic heart valves (72). Shortened platelet survival which occurs during the postoperative

state is, however, normalized by aspirin (32).

Aspirin may influence hemostasis and thrombosis in ways distinct from its antiplatelet effects. Prolonged aspirin ingestion can result in a minor prolongation of the prothrombin time by unknown mechanisms (131). Enhanced fibrinolytic activity occurs following aspirin ingestion (101) and a recent report suggests that aspirin stimulates fibrinolytic activity stemming from cellular elements in the blood (110). The significance of these findings, and their contribution to the antithrombotic effect of aspirin, is unknown.

In animals, aspirin treatment reduces the mass of arterial thrombi and improves patency of arterial grafts, shunts, and endarterectomized vessels in a variety of experimental situations (38,54,87,132). Aspirin has also been shown to inhibit platelet aggregation in the cerebral microcirculation in experimental models (141). Experimental venous thrombosis has been reported to be little affected by aspirin in conventional dosages (126).

Clinically, aspirin may be a useful agent in preventing myocardial infarction and transient cerebral ischemic attacks. This view is based on retrospective data and anecdotal experiences (18,43,75), and data from multicenter trials are needed before aspirin therapy can be recommended in these disorders. Aspirin, despite its inability to normalize platelet survival, has been reported to significantly reduce the incidence of thromboembolism from prosthetic heart valves when used in conjunction with warfarin (5). It has also been shown to reduce thrombotic deposits on dialysis membranes (92). In orthopedic patients, aspirin has been demonstrated effective in reducing the incidence of venous thromboembolism and can be recommended for prophylaxis (74,82,145). In general surgical patients, the data are more conflicting and alternative measures, such as low-dose heparin, are of proven merit.

Aspirin was found ineffective in improving exercise tolerance, chest pain thershold and ECG changes associated with angina pectoris (57). It has also been found not to reduce the frequency of diminished pulses following arterial catheterization (55). Aspirin was also found without benefit in halting the progression of childhood glomerular disorders (167).

Although a single small dose of aspirin produces measurable effects on platelet function tests (160), the dose necessary to achieve optimal antithrombotic effects is unknown. Dosages in clinical trials have varied considerably. At present, it seems reasonable that 2 tablets taken twice daily (650 mg B.I.D.) should produce any antithrombotic effect to be realized from the drug. Much less may be necessary.

Clofibrate.

Clofibrate exerts an effect on platelet function independent of its effects on plasma lipids. It has been reported to have a mild suppressant effect on platelet aggregation induced by ADP, epinephrine and collagen, to reduce platelet retention in glass-bead columns and to prolong the bleeding time (27,63,121,139). Unlike aspirin, the drug appears to have little effect on the platelet release reaction. Although the mechanism of action is unknown, it has been suggested that clofibrate produces membrane alterations that decrease the sensitivity of the platelet to aggregating agents (24). Clofibrate has also been reported to increase blood fibrinolytic activity (27).

In clinical studies, two trials have reported clofibrate beneficial in reducing the incidence of myocardial infarction (65,134). However, a better-designed trial could not substantiate this finding (34). Patients with type II familial hyperlipoproteinemia have abnormally reactive platelets and Carvalho et al (24) have reversed this abnormality by treatment with clofibrate. Whether this will reduce the incidence thrombotic complications in this group of patients in unknown.

Dextran.

Dextran is a partially hydrolysed glucose polymer from the bacteria Leuconostoc mesenteroides. There are two preparations for clinical use, dextran 70, or "clinical dextran" (average molecular weight 70,000) and dextran 40, or "low molecular weight dextran" (average molecular weight 40,000). The two preparations are not distinct; the lower molecular spectrum of one overlaps the upper molecular spectrum of the other (8). Although the low molecular weight dextran preparation is more rapidly excreted, about 20% of the molecules have a molecular weight in excess of 50,000 and are above the renal threshold. They must be metabolized, and the repeated administration of low molecular weight dextran leads to the accumulation of an effective blood level (6). Normal platelet function is present when dextrans are added to citrated blood in concentrations comparable to those achieved in vivo (178). However, 4-8 hr following in vivo infusion of either preparation, there are variable decreases in platelet adhesiveness, defective platelet aggregation to ADP and collagen, and impaired platelet release (13,21,36,178). This has led to the view that the antiplatelet effect of dextran stems from its ability to complex with plasma proteins, some of which may be necessary for platelet aggregation (21). The antithrombotic effect of dextran may also be related to its ability to cause defective fibrin polymerization (119). This may result in an unstable clot susceptible to lysis.

Experimentally, dextran can reduce arterial and venous thrombosis in animal models. Clinically, it is effective in preventing venous thrombosis in a variety of postoperative patients (33). It has been utilized following arterial reconstructions to prevent thrombosis of grafts and endartectomized vessels but there are no controlled data to confirm its benefit for this purpose.

Dipyridamole.

The precise mechanism of action of dipyridamole on platelets in unclear. It inhibits the uptake of glucose by platelets and this may interfere with platelet metabolism (37). This is suggested by its ability to inhibit the ADP-induced burst of CO_2 production by platelets (37). Dipyridamole also is a phosphodiesterase inhibitor (104) and results in elevated levels of cyclic AMP in platelets, a process inhibitory to aggregation (146).

Dipyridamole and similar pyrimido-pyrimidine compounds impair ADP, collagen, epinephrine, and thrombin-induced platelet aggregation (37,45,76). In contrast to aspirin, dipyridamole has its most pronounced effect on the first rather than the second (release-dependent) phase of aggregation. To a lesser extent than aspirin, it interferes with platelet release (37). This drug also reduces platelet retention in glass-bead columns and depressed platelet factor 3 availability (76,136). In in vivo tests, dipyridamole has little or no effect on the bleeding time in man but does normalize platelet survival shortened by prosthetic heart valves (72). In experimental animals, dipyridamole reduces thrombus deposition in extracorporeal shunts, in intimectomized arteries, and at cut ends of small arteries (37,38,48). It has little effect on experimental venous thrombosis in conventional dosages (126). Dipyridamole has also been shown to reduce microembolic phenomena associated with experimental cardiopulmonary bypass (137).

In clinical situations, dipyridamole appears most promising in preventing thromboembolic complications associated with prosthetic heart valves. Sullivan et al. (162,163) significantly reduced the incidence of valve thromboembolism in a trial comapring dipyridamole and placebo. All patients were anticoagulated with warfarin. By inference, dipyridamole may hold promise in preventing thromboembolism associated with other prosthetic arterial devices. The drug may also be helpful in ameliorating rejection of allograft kidneys (96) and other organs but more data are necessary in this area.

In controlled, clinical trials dipyridamole has been found ineffective in cerebrovascular disease (TIA) (2) and myocardial infarction (58). The design of these trials has been criticized and the final place of this drug in preventing these disorders awaits further trials. Dipyridamole has been conclusively shown to be ineffective in preventing postoperative venous thromboembolism (20,145).

In contrast to aspirin, platelet function is impaired only while dipyridamole is in the circulation. Optimal dosages and dosage schedules to ensure sustained platelet inhibition are unknown. Dosages have varied in clinical trials but most investigators have employed 400-600 mg daily in 4 divided doses. The normalizing effect of dipyridamole on platelet survival shortened by prosthetic heart valves can be achieved by as little as 100 mg daily when administered as a single dose along with 12. g of aspirin (72). The potentiating effect of aspirin on dipyridamole may produce a more pronounced antithrombotic effect than either drug alone and this combination is being evaluated in several clinical trials (172).

Hydroxychloroquine Sulfate.

The antimalarial, hydroxychloroquine sulfate, also inhibits ADP-induced platelet aggregation (22). The exact mechanism of action is unknown and there are little data available on the *in vitro* and *in vivo* platelet inhibiting effects. The drug appears promising in prevention of venous thromboembolism. Carter and Eban (22,23) reported two randomized, controlled trials in general surgical patients and were able to demonstrate a significant reduction in venous thrombosis. More recently, Chrisman *et al.* (29) in a preliminary report documented a significant reduction in venous thrombosis in patients with hip fractures or undergoing major orthopedic procedures. Confirmation of these optimistic reports by well-designed clinical trials is necessary before this drug can be recommended. It seems important to evaluate this drug in arterial thromboembolic disorders since there are no published data in this area.

Sulfinpyrazone.

Like aspirin, sulfinpyrazone appears to alter platelet function primarily through inhibition of platelet release (127,195). Similar mechanisms may be operative in that sulfinpyrazone has been shown to inhibit platelet prostaglandin synthesis (4). The drug produces variable changes in *in vitro* platelet function. Therapy with 800 mg daily reduces retention of platelets in glass-bead colums but produces no changes in platelet aggregation to ADP and collagen (176). *In vivo*, sulfinpyrazone lengthens platelet survival in experimental

animals and in humans (114,115). Effective dosages employed in most clinical trials have ranged from 600 to 800 mg daily in 3-4 divided doses.

Clinically, sulfinpyrazone has been shown to reduce the frequency of amaurosis fugax and transient cerebral ischemic attacks in a double-blind, crossover trial conducted by Evans (52). A larger, multicenter trial is currently in progress which should ultimately establish the efficacy of sulfinpyrazone in cerebrovascular disease (172). In a single report, sulfinpyrazone normalized platelet survival shortened by prosthetic heart valves (176). It may also reduce the incidence of valve-related thromboembolism but this is unproven. Sulfinpyrazone has been shown to reduce the frequency of arteriovenous shunt thrombosis in patients on chronic hemodialysis (88). In cases of idiopathic recurrent venous thrombosis associated with shortened platelet survival sulfinpyrazone may be useful in preventing attacks (158). The drug is currently being evaluated for the prevention of myocardial infarction, but no data are available.

CONCLUDING REMARKS

The experience cited above establishes the role of platelets in the pathogenesis of a variety of thromboembolic disorders. Despite advances in several areas, many problems remain. The relevance of platelet-function testing to thromboembolic disorders needs clarification. Whether the association of enhanced platelet function and the above disorders represents cause, effect, or nonspecific accompaniment is unknown. The concept of identifying individuals at risk by platelet function testing is attractive but unproven. Whether such individuals would benefit from prophylactic antiplatelet therapy is also unknown. For treatment of most established thromboembolic disorders as well as prophylaxis, the place of antiplatelet drugs is not established. Whether this form of therapy is superior to conventional treatment, adjunctive, or of no benefit is not resolved in most instances. Also, the most appropriate antiplatelet drug (or combination of drugs) in what dosages for specific disorders remains unclear. Well-designed, prospective clinical investigation, although cumbersome and time-consuming, will be necessary to answer most of these questions. A final problem area concerns basic research into the pharmacology of platelet inhibition. It is probable that the ideal antiplatelet agent remains undiscovered. Currently, several investigators are looking into the possibility of manipulating prostaglandin metabolic pathways in hopes of specifically blocking thromboxane generation while allowing production of metabolites inhibitory

towards aggregation. This exciting appraoch and other endeavors into the biochemical basis of platelet function will lead to the discovery of new antiplatelet agents.

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AD-A092 838 ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND FORT D--ETC F/G 6/5
MICROAGGREGATES: EXPERIMENTAL AND CLINICAL ASPECTS - SYMPOSIUM --ETC(U)
JUN 80 L K KOZLOFF, R J PORTER

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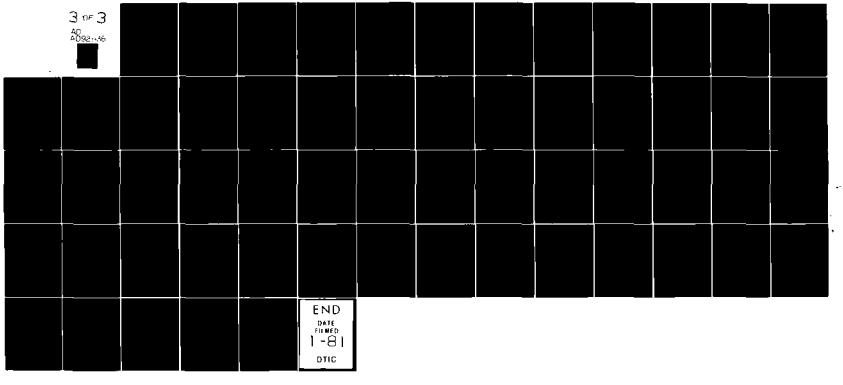

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CLINICAL SYNDROMES OF PLATELET HYPERAGGREGATION

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> "A 30 cm long thrombus which produces sudden death following embolisation from the deep veins of the legs is just as much a thrombus - in the sense that it kills by obstructing blood flow - as is a small platelet aggregate adhering to a ruptured atherosclerotic plaque in a coronary artery."
>
> Duncan P. Thomas (1)

The platelet has a unique role in hemostasis. It is peculiar in that it performs not only a mechanical function by forming platelet plugs but also a dynamic function by releasing various phospholipid compounds which may trigger or accelerate the coagulation mechanism. In addition, platelet aggregates may serve as nidi to which clotting factors may adhere and become activated. As mentioned by Thomas (1), the vital role of platelets in hemostasis has been known for many years. In his review, he credited Bizzozero with having been the first to demonstrate that platelets are the first formed elements to accumulate at the site of vessel injury.

Since then, a great deal of knowledge regarding platelet function has been gained. Teliologically, the primary role of platelets has been thought to be one of providing host defense in the face of vascular injury. It does so by a series of reactions starting with adhesion of platelets to the site of vessel injury. The second stage is one of aggregation of platelets to one another. This is followed by release of various constituents which augment platelet aggregation; the so-called phase of secondary aggregation. At this point, a preamble platelet plug has been formed. If not deaggregated or fragmented, this plug is made impermeable by incorporation of fibrin into its latticework.

While the above mechanisms are generally protective of the host, there is now considerable evidence to suggest that these mechanisms may at times proceed to the detriment of the host. Many methods of studying these mechanisms have been devised including measurements of bleeding time, clot retraction, adhesion to glass, screen filtration pressures, platelet factor 3 availability, platelet phagocytosis, platelet uptake of various substances and platelet aggregation (2,3). While platelet

survival is not a specific test of platelet function, its study promises to provide useful information regarding the role of platelets in various thrombotic processes.

At present, studies of platelet aggregation and survival provide the most convincing and reproducible evidence of altered platelet physiology in disease states. Tables 1 and 2 outline some of the conditions in which these studies have been abnormal.

Our own efforts have been primarily directed to the study of platelet aggregation in several categories of patients and will be outlined below.

TABLE 1. Partial list of conditions associated with shortened platelet survival.

1. Coronary artery disease
2. Prosthetic heart valves
3. Post operative state
4. Recurrent venous thrombosis
5. Widespread neoplasia
6. Bacteremia
7. Thrombocytopenic purpura
8. Hemolytic uremic syndrome
9. Vasculitis
10. Defibrination syndrome
11. Arterial prostheses
12. Hemodialysis fistulae
13. Hemodialysis shunts
14. Aortocoronary bypasses

TABLE 2. Partial list of conditions associated with enhanced platelet aggregation.

1. Transient ischemic attacks
2. Idiopathic recurrent deep venous thrombosis
3. Acute myocardial infarction
4. Stable angina
5. Amaurosis fugax
6. Nephrotic syndrome
7. Diabetes mellitus
8. Hyperbetalipoproteinemia
9. Acute arterial insufficiency
10. Arteriovenous thrombotic disorders

MATERIALS AND METHODS

Fifty-four patients with arterial thromboses only (20 patients), venous thromboses only (17 patients), or combined arterial and venous thromboses (17 patients) had determinations of spontaneous, epinephrine-induced, and adenosine diphosphate-induced platelet aggregation. Criteria for inclusion in the arterial thrombosis only group were: 1) age less than 30 with one or more arterial thromboses; 2) age greater than 30 with multi-system arterial thromboses, i.e., cerebrovascular accident and/or myocardial infarction plus a peripheral arterial thrombosis occurring at different times; 3) age greater than 30 with two or more temporally separated peripheral arterial thromboses. Criteria for inclusion in the venous thrombosis only group were: 1) two or more episodes of documented venous thrombosis with or without pulmonary embolism; 2) absence of known antecedent cause or association such as extremity trauma, malignancy, or birth control pills. To be included in the combined arterial and venous thrmobosis group, patients had to have had at least one arterial and one documented idiopathic venous thrombotic episode. Patients were excluded if studied while taking or within 3 weeks of having taken antigoagulant- or platelet-active drugs, if they had a malignancy, if studied during or within 3 weeks of an acute thrombotic event, or if studies less than 3 weeks after an operation.

Twenty volunteers had the same studies performed to serve as controls. Some controls were in the arteriosclerotic age group but none had had a thrombotic event.

In a separate study, 19 patients with all stages of colon malignancy had similar studies performed. All were studied before treatment with chemotherapeutic agents, and all were asymptomatic with reference to bleeding or thrombosis. None were taking or had recently taken platelet-active drugs.

The technique of platelet aggregometry was as described by Born and Cross (4). As part of our screening battery, all patients and 32 controls had each of the tests outlined in Table 3 and determinations of fibrin monomer by the method of Gurewich and Hutchinson (5).

RESULTS

Patients with arterial thromboses tended to be older (mean age = 51.7 years) than patients with combined arterial and venous thrombotic disorders (mean age = 45.4 years) or patients with only venous thrombotic disorders (mean age = 40.1 years). There were more females in the group with venous thromboses only (52.9%) than in either the group with arterial thromboses

TABLE 3. Coagulation battery for patients with thrombotic disorders.

1.	General screening tests
	Prothrombin time
	Activated partial thromboplastin time
	Thrombin time
	Fibrinogen
	Platelet count
2.	Tests for individual factors
	Factors II, V, VII-X, VIII IX, XI, XII
3.	Platelet function
	Epinephrine-induced platelet aggregation
	Adenosine diphosphate-induced platelet aggregation
	Collagen-induced platelet aggregation
	Platelet factor 3 availability
4.	Tests for fibrinolysis and antithrombosis
	Fibrin split products
	Progressive antithrombin activity

only (20%), or the group with combined arterial and venous thrombotic disorders (17.6%). These differences are reflected in Table 4. The data derived from the platelet aggregation studies are illustrated in Figures 1 and 2 and shown graphically in Tables 5 and 6. All patient groups had platelet hyperreactivity both to epinephrine and adenosine diphosphate when compared with controls.

Spontaneous platelet aggregation was noted only in patients with combined arterial and venous thrombotic disorders. The data from these three patients are not reflected in the geometric means tabulated since aggregation occurred at zero concentrations of reagents. Results were already highly statistically significant without further manipulations of the transformed data.

Compared with controls, factor VIII and XI levels were significantly elevated in all groups. Factor IX levels were elevated in all groups but the mean values (percentage of normal ± SEM) differed from the mean value for controls (103.6 ± 7.8) and in patients with combined arterial and venous thrombotic disorders (133.5 ± 17.8). These data are fully elaborated in Table 7.

Similarly, fibrinogen levels (milligrams/100 ml ± SEM) were significantly elevated in patients with arterial thromboses only (455.0 ± 34.3) and in patients with combined arterial and venous thromboses (423.0 ± 30.4). Although the mean value of

TABLE 4. Sex and age distribution of patients studies.

Category	Number	Mean age, yr	Sex (m/f)
Control	32	35	23/9
Arterial thrombosis	20	52	16/4
Venous thrombosis	17	40	8/9
Combined	17	45	14/3

TABLE 5. Minimum concentrations (geometric means) of epinephrine inducing full aggregation (thrombosis patients).

	μ M Concentration
Control	0.80
Arterial	0.08[1]
Venous	0.32[2]
Combined	0.09[1,3]

1/Significantly different than mean value for controls at $p<0.001$.
2/Significantly different than mean value for controls at $p<0.05$.
3/Excludes patients with spontaneous aggregation (see text).

TABLE 6. Minimum concentrations (geometric means) of ADP including full aggregation (thrombosis patients).

	μ M Concentration
Control	2.96
Arterial	1.14[1]
Venous	1.75[2]
Combined	0.95[1,3]

1/Significantly different than mean value for controls at $p<0.001$.
2/Significantly different than mean value for controls at $p<0.05$.
3/Excludes patients with spontaneous aggregation (see text).

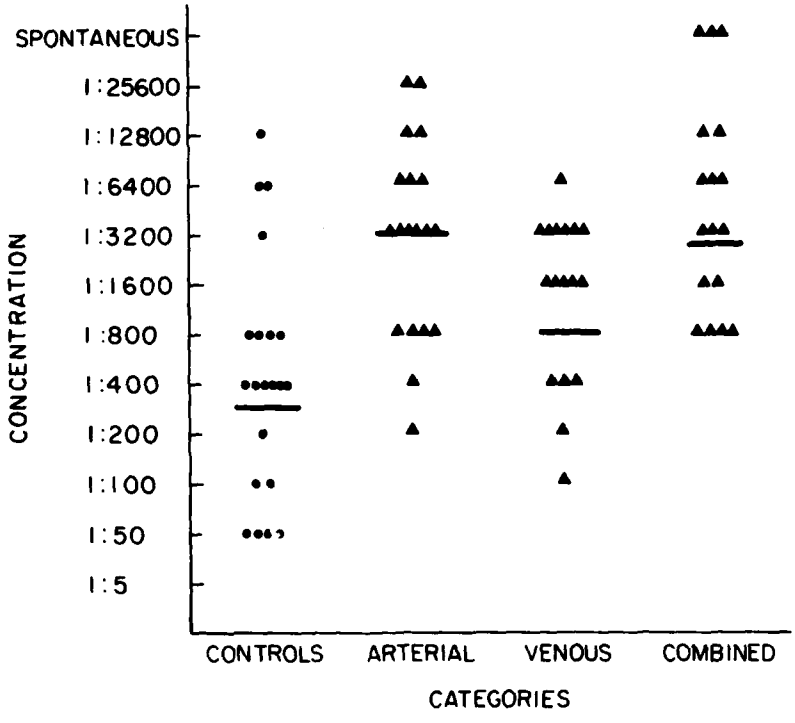

Fig. 1. Distribution of epinephrine induced platelet aggregation responses. Horizontal bars refer to geometric mean concentrations of reagent causing full aggregation.

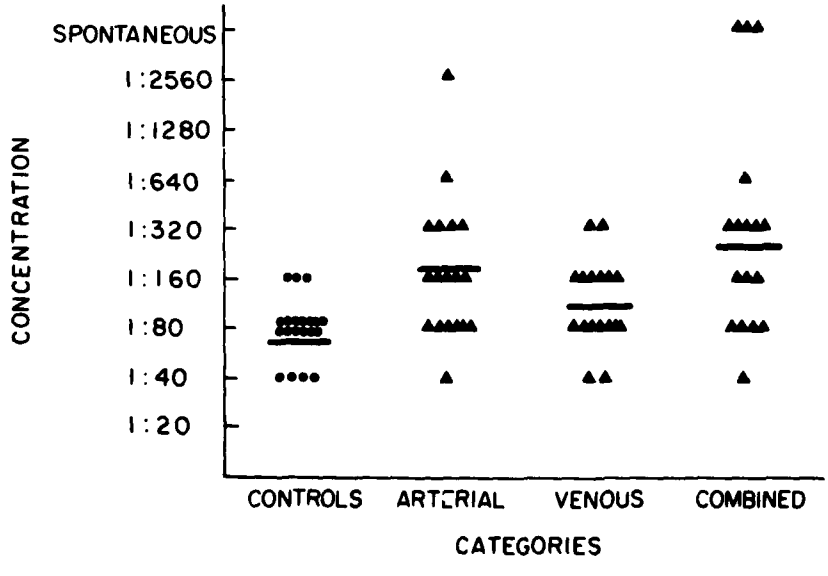

Fig. 2. Distribution of ADP induced platelet aggregation responses. Horizontal bars refer to geometric mean concentrations of reagent causing full aggregation.

TABLE 7. Factor levels in patients with thrombotic disorders.

Category	Factors, % of normal ± SEM		
	VII	IX	XI
Controls	105.4 ± 5.6	103.6 ± 4.7	108.4 ± 5.3
Arterial	146.3 ± 10.5	123.2 ± 7.8	136.2 ± 14.9
Venous	123.3 ± 7.3	118.2 ± 5.6	139.5 ± 8.2
Combined	148.2 ± 12.7	133.5 ± 17.8	146.8 ± 15.9

1/Significantly different than control value at $p<0.001$.
2/Significantly different than control value at $p<0.05$.
3/Significantly different than control value at $p<0.005$.
4/Significantly different than control value at $p<0.01$.

361.8 ± 27.4 found in patients with venous thromboses only was higher than the value of 306.3 ± 20.2 in controls, the difference was not statistically significant. Regarding antithrombin III activity, 36,8% of patients with arterial thromboses only, 17.6% of patients with venous thromboses only and 26.7% of patients with combined arterial and venous thromboses had depressed antithrombin III activity.

Sixty percent of patients with arterial thromboses, 64.7% of patients with venous thromboses, and 31.7% of patients with combined arterial and venous thromboses had shortened activated partial thromboplastin times. The mean values differed significantly from the mean value for controls only in the group with venous thromboses ($p<0.005$). The results of other studies performed are not reported for reasons previously stated (6).

In patients with colon malignancy, 8 of the 19 (42.1%) had enhanced platelet aggregation evidenced by full aggregation upon addition of 0.16 μ mole of epinephrine or less. Six of the 19 patients had advanced malignancies. In this subgroup, four had circulating fibrin monomer. Only three patients with malignancy had spontaneous platelet aggregation and they were in this subgroup. The fibrinogen level in this group of patients was 484.2 mg/m., and the average fibrin split product level was 28.8 μg/ml. These data are outlined in Table 8.

DISCUSSION

Prominent coagulation abnormalities exist in patients with histories of arterial, venous and combined arterial and venous thrombotic disorders. Whether or not these particular abnormalities actually cause thromboses or are simply associated

TABLE 8. Coagulation values in patients with malignancy.

	Normal	All patients (n=19)	Advanced Malignancy
VII, % of normal	100	127.2	176
Fibrinogen, mg/100 ml	300	400.5	484.2
A.P.T.T, sec	36	29.4	-
Fibrin split products, µgm/ml	<16	38.8	28.8

findings in patients with thrombosing tendency remains unanswered. At the very least, they serve as important biological markers in patients who have previously had thrombotic episodes and who are presumably predisposed to more episodes.

Similar abnormalities exist in patients with malignancies. Other authors have described a variety of coagulation abnormalities in patients with malignancies. Edwards (7), in 1949, described six personal cases of migratory thrombophlebitis associated with visceral malignancy and credited Trousseau with the first description of the disease (9). Edwards, however, reported on laboratory studies. Amundsen et al. (8), in 1963, reported elevated factor VIII levels and accelerated thromboplastin generation in patients with a variety of malignancies. As in our patients, Sun and coworkers (9) have pointed to evidence of a chronic consumptive coagulopathy in patients with malignancy. The finding of enhanced platelet aggregation in patients with malignancy is not surprising in view of its frequency in patients with other types of chronic and recurrent thrombotic problems as reported here. Davis and coworkers (10) have also noted increased platelet aggregation in patients with malignancy.

Controversy continues regarding whether or not separate mechanisms are involved in arterial versus venous clotting. It has long been thought that platelets play a dominant role in arterial thrombosis though they are relatively unimportant in venous thrombosis, in which case the participation of activated coagulation factors and fibrinogen play the dominant role (11).

It has been suggested that the concept that arterial and venous thromboses occur through significantly different pathogenic mechanisms may be an oversimplification (12). Our data are supportive of that concept in that we were able to demonstrate significant abnormalities in platelet reactivity and clotting factors in all groups. In general, these abnormalities were more marked in patients with arterial and combined arterial and

venous thrombotic disorders than in patients with purely venous thrombotic disorders. The importance of coagulation abnormalities and abnormal platelet function in patients with myocardial infarctions, transient ischemic attacks, cerebrovascular accidents and peripheral arterial thromboses has been stressed by other authors (13-16). Of interest is the fact that O'Brien and coworkers (17) found increased antiheparin activity (presumably platelet factor 4) in patients with myocardial infarction. They found similarly increased levels in patients with intermittent claudication but no history of myocardial infarction suggesting that coagulation abnormalities may presage a thrombotic event.

Of particular interest is the mounting evidence suggesting an important role of platelets in venous thrombosis (18-20). As a corollary, there is a growing body of evidence suggesting that platelet active drugs may prevent venous thromboses (21,22). Our data support the concept that platelets have a major role in both arterial and venous thrombosis since platelet hyperaggregation was noted in all groups of patients.

Platelet hyperreactivity was more intense in patients with arterial and combined arterial and venous thrombotic disorders than in patients with purely venous thrombotic disorders. Spontaneous aggregation was noted only in three patients with combined arterial and venous disorders. It is of interest that of these three patients, two have had further thrombotic episodes since the time of the study. Neither was on treatment at the time of development of the thrombotic episode. One developed a stroke associated with an arteriogram and the other died at age 41 of myocardial infarction.

Other authors have noted spontaneous platelet aggregation in patients with recurrent venous thromboses (23). We have observed spontaneous platelet aggregation in patients with malignancy and in patients with acute arterial and venous thromboses. Presently, we regard spontaneous platelet aggregation as a distinctly pathologic condition worthy of treatment.

In keeping with our earlier reports (6,24), we have continued to treat patients with thrombotic disorders who have specific laboratory abnormalities on the basis of the predominant abnormality. If there is evidence of platelet hyperaggregation, we administer acetylsalicylic acid (aspirin, 600 mg daily) as the primary drug. Because of a sensitivity to aspirin in some patients, and failed clinical responses in a few patients with transient ischemic attacks (not included in this series), we have added sulfinpyrazone to our armamentarium. The results of others who have used this drug for its platelet-active qualities suggest that it may be highly effective in preventing thromboembolism (12,25).

We administer coumadin in patients with antithrombin III deficiency and/or factor abnormalities. When there are combined platelet and factor abnormalities, we have preferentially treated the platelet abnormalities so that few patients actually end up taking coumadin. While this approach is logical, our series is too small to judge whether or not these drugs will actually decrease the incidence of thromboses. Anecdotally, our early results have been promising (7). Several large trials comparing the effectiveness of various platelet active drugs are now underway (26).

These studies cannot be considered definitive at this time. Other authors have cited the problems associated with using analysis of individual coagulation factors to diagnose hypercoagulability and the need for doing activated factor assays (27). Analysis of individual coagulation factors, however, become much more meaningful when done as part of a composite battery of tests as we have done them and when correlated with clinical events. They are an important step in a progression of studies that will, hoepfully, help explain the mechanisms involved in inappropriate thrombosis. Moreover, it is extremely important to study platelet function as well as coagulation in screening for laboratory confirmation of thrombosing tendency. Rather than selecting treatment on the basis of whether patients have arterial or venous thromboses, it seems more logical to treat patients on the basis of whether they have primarily platelet or primarily coagulation abnormalities.

SUMMARY

Prominent abnormalities of platelet function, coagulation factors, fibrinogen levels, activated partial thromboplastin times, and antithrombin III activity can be identified in patients with arterial thromboses, venous thromboses and combinations of the two. Similar abnormalities occur in patients with malignancy but evidence suggests a process of consumptive coagulopathy in patients with malignancy not seen in other types of patients. These studies may help explain some of the mechanisms involved in patients with thrombotic disorders and may be useful in selecting appropriate prophylaxis or therapy.

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THE STORAGE LESION OF BLOOD: ROLE IN MASSIVE TRANSFUSION AND MICROAGGREGATE FORMATION

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Microaggregates

The administration of an artificially preserved biological such as blood plus complex aspects of shock produced a summated integrated system in which homeostatic mechanisms normalize the endogenous metabolic aberration, alter the administered blood to buffer its acid, normalize its metabolically inactive red cells, warm its enzymatic systems and produce a functional entity which is comparable to that shed during injury (Fig. 1). It is difficult to assess the role of microaggregates in transfusion because the shock insult is associated with production of microaggregates which contribute to pulmonary failure. Moreover, the administration of microaggregates in preserved blood are to some degree the function of the storage interval of blood and the number of units administered. Although it is universally stated that the clinical situation most benefitted by filtration of blood is massive transfusion, in reality blood of extended shelf life with the largest quantity of particulate matter is usually administered early during massive transfusion. Subsequent depletion of blood bank resources results in the magnitude of the transfusion being with blood of relatively short shelf life. Moreover, in military situations where walking donor blood is frequently used, fresh blood will frequently be available which has minimal microaggregates. In addition, the filtration of blood, even by the new filters, removes some platelets which may be of prime importance in the reversal of the bleeding problem which initiated transfusion.

Endogenous sources of microaggregates

One of the intermediate mechanisms responsible for the respiratory distress syndrome is thromboembolism. In each clinical situation there are many variables, such as existing state of health, duration of insult prior to treatment, magnitude of resuscitation, extent of hyperoxia administered to the pulmonary system, etc., which influence pulmonary function. Dead or devitalized tissue results in fragments of collagen and fat entering the blood stream directly. Not only are these fragments direct embolic phenomena, but they are important activators of

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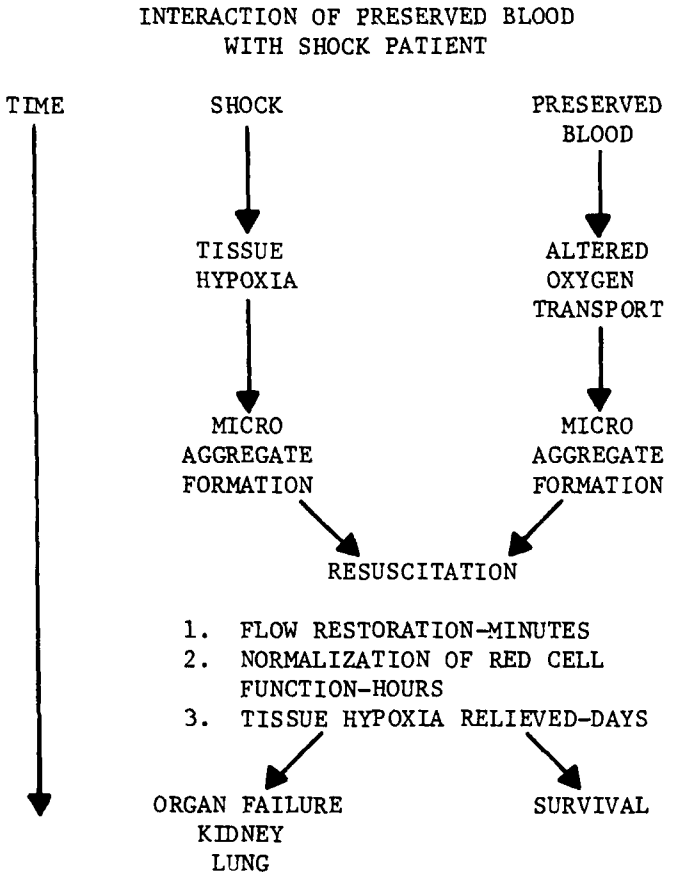

Fig. 1. Schematic representation of the formation of microaggregates in liquid-preserved blood and endogenous formation in patients with shock.

the clotting mechanism. Intravascular aggregation of the platelets is reversible under most circumstances. If, however, fibrin is incorporated into the aggregation, the platelet mass is stabilized and disaggregation will not occur. Permanent obliteration of the pulmonary microcirculation may occur in such situations.

Tissue embolism was one of the initially recognized causes of pulmonary failure; particles of neutral fat (fat embolism) and bone marrow may be found in lungs immediately after injury and after cardiopulmonary resuscitation. These particles may mechanically obstruct vessels and produce an embolic phenomenon followed by an inflammatory response. Fat embolism, however, has been an infrequent occurrence since prompt resuscitation and precise monitoring of the patient with fractures has been our

practice. Our current opinion is that fat does not simply mechanically obstruct the pulmonary microcirculation; it is suspected that fat initiates intravascular coagulation.

Sludging of blood in areas of injury was demonstrated many years ago by Knisely (18). After resuscitation "sludged" blood and products of anaerobic metabolism are released into the circulation and may cause further activation of the clotting mechanism with microembolism.

Platelet aggregation are readily demonstrated in the blood following soft tissue trauma or prolonged shock. In studies of hemorrhagic, traumatic or endotoxin shock, emboli are present in the microvasculature of the bowel and injured tissue. If transfused blood is administered after shock is induced, showers of platelet aggregates enter the blood stream and are filtered out by the lung, presumably leaving damaged lung units. During the episodes of platelet showers, platelet counts fall as platelets sequentially sequester in the lungs. With successful resuscitation, platelets rapidly disaggregate and platelet counts return to normal. If the shock insult is repeated after this sequence of events, increased pulmonary vascular resistance and death occur as a consequence of a further deposition of platelets. Studies by Berman et al. (12) have documented intraalveolar hemorrhage which resulted when venous drainage of the lung of an injured thigh following gunshot wounds released platelet aggregates into the pulmonary circulation. In studies of standard lung trauma and fracture to the hing legs of dogs, Saggau et al. (20) reported that neither the hemorrhagic shock nor the bone trauma insult produced death. A combination, however, of hemorrhage plus fracture resulted in uniform lethality. Histological examination of these animals revealed microemboli in the lung units.

MATERIAL AND METHODS

Eight units of whole blood were drawn at Irwin Memorial Blood Bank. Four of the units were divided into quadpacks and four units were maintained as whole blood in citrate phosphate dextrose (CPD) preservative. Standard National Institutes of Health citrate phosphate dextrose preservative was used. Blood was stored between sampling intervals at 4°C in a refrigeration unit monitored by Irwin Memorial Blood Bank of the San Francisco Medical Society.

Packed red blood cells were collected by Irwin Memorial Blood Bank into Fenwal bags and divided immediately into tripacks for removal of plasma. Instead of reconstituting into a single packed cell unit, they were left in tripacks so that it would not be necessary to agitate all three bags during the sampling process. Sampling was done with a syringe from the end of the tubing used

to introduce blood into the bag. Care was taken to keep bags in an upright position between sampling. Sampling was done every 2 days for a 3-week period. All blood was stored in a refrigeration unit at 4°C and monitored by Irwin Blood Bank to comply with standard blood bank procedures.

Samples from eight different variables were taken on alternate days through the majority of the storage life of 21 days. Diphosphoglycerate was measured using the Sigma kit. Adenosine triphosphate and lactate were measured using Calbiochem kits. pH was measured using a Radiometer electrode. Saturation and hemoglobin were measured on the Instrumentation Laboratories Co-oximeter 182. Calcium and phosphate determinations were performed on the autoanalyzer.

Cutter bags were used for the whole blood study with the exception of one Fenwal bag. Results were expressed as absolute values and as percentages of the initial values and matched according to time and storage (Tables 1 and 2). Comparisons between the two groups were performed using "Students" t-test or when variances differed, Cochran's weighted t prime was used. Differences within groups were assessed by analysis of variance. Regressions were performed to evaluate rates of change and covariance analysis applied for comparing slopes between the groups.

Group samples were found to be free of skewness and positive kurtosis.

RESULTS

Diphosphoglycerate (2,3 DPG)

Diphosphoglycerate as the major human intraerythrocytic organic phosphate is lost during all preservation procedures to some degree (Fig. 2). In whole blood preserved in citrate phosphate dextrose, 2,3 DPG was significantly below the baseline by day 7, representing a loss of approximately 50%. By day 18 of storage, less than 1 μ mole/gHb of 2,3 diphosphoglycerate remained, representing approximately 7% of the initial value.

The slower rate of deterioration during week 2 of storage is the advantage obtained in citrate phosphate dextrose preservative over acid citrate dextrose (ACD) preservative. Maintenance of oxygen transport capabilities is better during the initial week but falls to comparable levels by the end of the storage life.

TABLE 1. CHANGES IN WHOLE BLOOD AND PACKED CELLS DURING STORAGE IN CPD PRESERVATIVE

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	WHOLE BLOOD									days on which values differ significantly from day 0 ($p<0.05$)
	0 days	2 days	4 days	7 days	9 days	11 days	14 days	16 days	18 days	
2,3 DPG μ moles/gHb	11.7±1.1	11.7±1.4	12.0±1.9	8.2±1.6	5.7±1.2	3.8±0.9	2.0±0.9	1.6±0.6	0.9±0.3	
%	100	100.1±9.9	102.2±14.5	70.5±13.8	48.8±11.3	32.1±8.1	17.5±8.0	11.6±7.2	7.7±2.3	7+
ATP μ moles/gHb	3.41±0.77	3.83±0.65	3.74±0.64	3.22±0.49	3.09±0.97	3.09±0.32	2.75±0.75	2.74±0.45	2.58±0.53	
%	100	118.1±37.0	116.4±37.7	98.3±25.3	97.4±41.4	96.8±31.4	85.9±26.4	85.7±25.9	84.7±32.4	NS
Lactate μ moles/gHb	13.8±2.1	33.9±4.4	52.8±4.0	77.0±9.1	91.3±10.3	108.1±14.8	124.1±16.7	136.9±20.8	145.6±16.6	
%	100	250±41	391±66	565±58	670±74	794±118	937±118	1065±118	1140±156	2+
pH units	7.0±0.08	6.96±0.05	6.91±0.05	6.86±0.05	6.81±0.05	6.8±0.05	6.73±0.03	6.60±0.02	6.68±0.02	
% (day 0-6.0)	100	96.8±10.9	91.9±10.6	86.9±10.9	82.4±8.7	80.3±8.0	75.4±8.6	71.0±7.7	69.5±8.6	7+
SO_2 %	50.9±12.2	49.7±8.4	53.0±11.0	56.5±16.7	54.8±17.0	64.6±21.1	68.3±21.0	69.8±22.3	72.0±20.8	
% day 0	100	105.3±39.9	111.4±40.4	122.9±61.9	114.5±45.6	136.4±57.8	148.7±76.2	150.0±71.3	159.6±76.8	11+
Ca mg/100ml	7.88±0.35	7.79±0.24	7.78±0.19	7.96±0.17	7.88±0.32	8.1±0.23	8.08±0.3	8.12±0.08	8.23±0.17	
%	100	99.0±4.8	97.9±4.0	101.4±4.3	100.1±4.5	103.4±5.6	102.7±5.4	103.2±6.0	105.8±7.3	NS
Pi mg/100ml	10.16±0.83	9.79±0.83	9.63±1.09	12.68±3.21	11.19±2.2	13.45±2.37	13.60±3.27	13.44±3.33	14 (n=1)	
%	100	96.3±4.1	95.3±6.4	130.0±25.3	111.3±25.6	128.3±15.7	132.7±36.9	132.9±30.6	137.8	7,11

TABLE 2. CHANGES IN WHOLE BLOOD AND PACKED CELLS DURING STORAGE IN CPD PRESERVATIVE

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PACKED CELLS

	0 days	2 days	4 days	7 days	9 days	11 days	14 days	16 days	18 days	days on which values differ significantly from day 0 ($p<0.05$)
2,3 DPG μ moles/gHb	13.4±3.5	14.1±2.8	12.2±1.4	9.5±3.4	7.3±2.6	5.2±0.8[1]	2.78±0.8	2.2±0.5	1.2±0.4	
%	100	106.6±9.7	93.3±12.6	69.9±10.2	53.7±8.6	39.6±7.0	23.4±4.7	16.6±3.8	9.4±2.5	7+
ATP μ moles/gHb	3.75±0.43	3.65±0.43	3.73±0.37	3.59±0.33	3.77±0.24[3]	3.56±0.17[1]	3.36±0.39	3.42±0.42[1]	3.05±0.66	
%	100	96.6±11.0	101.6±9.1	96.0±6.3	100.9±6.9	95.9±13.2	90.0±8.6	91.1±2.4	79.5±11.2	18
Lactate μ moles/gHb	18.6±2.8[3]	32.6±2.0	48.0±3.4	65.9±4.4[1]	85.6±9.5	103.3±11.9	112.1±7.1	122.4±5.3	132.4±6.2	
%	100	184.0±15.0[1]	254.6±23.8	358.2±41.6[1]	463.1±43.7[2]	541.9±66.4[2]	612.5±98.4[2]	666.3±76.1[3]	720.5±79.0[3]	2+
pH units	6.9±.05[1]	6.86±.04[2]	6.81±.05[2]	6.73±.06[3]	6.67±.04[3]	6.64±.03[3]	6.58±.04[3]	6.53±.03[3]	6.55±.04[3]	
% (Days 0-6)	100	95.2±2.1	89.8±2.2	80.2±2.5	74.1±8.7[1]	70.7±3.7[1]	64.0±3.6	58.6±1.6[1]	60.9±4.2	4+
SO_2 %	37.7±7.5	42.0±9.4	48.8±13.4	46.9±5.2	58.3±9.4	68.7±12.8	51.4±6.4	61.0±5.3	66.2±10.4	
% day 0	100	111.5±11.2	129.5±23.9	134.3±31.8	162.3±55.6	193.0±76.1	140.7±30.5	168.6±31.8	181.7±47.0	9,11,16
Ca mg/100ml	8.18±0.25	8	8.2±0.14[3]	8.34±0.17[3]		9.15±0.21[3]	9.18±0.19[3]	8.8[2]	9.25±0.10[3]	
%	100	96.4	100.4±3.7	102.5±3.7		112.8±5.4	114.0±5.4	106.0	113.2±4.2	11+
Pi mg/100ml	10.25±0.54	9.80	9.30±0.96	10.56±1.47	14.1	14.93±2.10	16.43±1.82	22	20.63±2.78	
%	100	96.1	92.9±9.2	100.3±12.9	137.3	143.8±20.1	157.7±18.5	215.7	201.2±25.1[1]	9+

[1]Denotes significant difference at 5% level
[2]Denotes significant difference at 1% level
[3]Denotes significant difference at 0.1% level

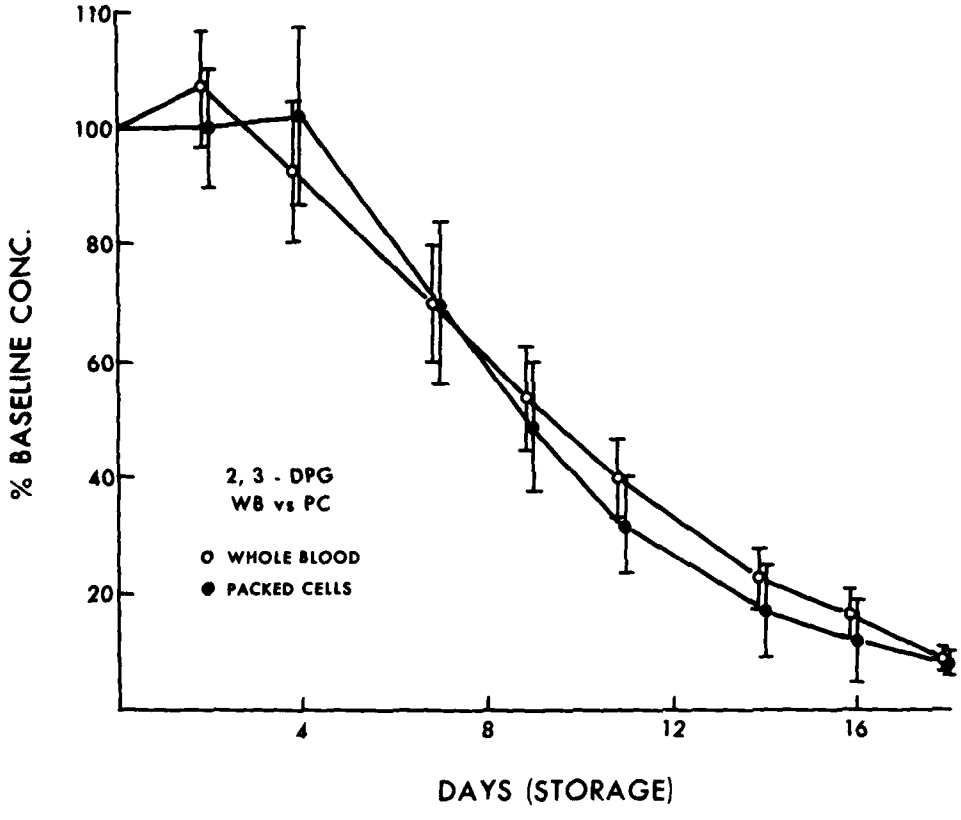

Fig. 2. Diphosphoglycerate deterioration during liquid perservation in CPD solution. By permission, Journal of Trauma, 1977.

Packed red blood cells had hematocrits ranging between 70-75% in all bags with the hemoglobin approaching 25. Equalization of this for comparative purposes was obtained by expressing the diphosphoglycerate levels in micromoles per gram of hemoglobin. The baseline value of diphosphoglycerate was 13.4 $\pm$ 3.5 μ moles/gHb. By day 7, a statistically significant drop in diphosphoglycerate levels had occurred to approximately 50% of the baseline level. By day 18 of storage, the values had lowered to 1.2 $\pm$ 0.4 μ mole/gHb.

The deterioration of diphosphoglycerate in packed cell preservation was identical with that of citrate phosphate dextrose (see graph and table). These studies would indicate that no difference in oxygen transport capability should occur based on the role of 2,3 DPG in altering hemoglobin-oxygen affinity.

Adenosine triphosphate

It has been shown that adenosine triphosphate levels correlate well with the posttransfusion red cell survival. Current criteria for allowing 21 days of storage are based on this proposition. For this reason preservatives have been assessed by the decay rate of adenosine triphosphate. As has been previously demonstrated by many authors, whole blood demonstrates no significant drop in adenosine triphosphate in citrate phosphate dextrose preservative during the entire period of the study. This would merely substantiate the prior findings of others that posttransfusion survival should be adequate.

In 1953, Gibson demonstrated that adenosine triphosphate levels were maintained throughout a 21-day period storage interval as packed cells. The significance of this finding was the ability of packed cells, following the initial blood donation, to be used at any time during a normal storage period. Our studies would indicate that adenosine triphosphate levels fell below normal by day 18 to a small degree, consistent with a 70% posttransfusion survival. These findings merely reconfirmed Gibson's prior work. One would expect that posttransfusion survival of packed cells during the normal 21-day storage interval would be satisfactory.

No statistically significant difference was found between adenosine triphosphate levels in whole blood or packed red blood cells. This should encourage the use of packed cells instead of whole blood.

Lactic acid

Lactic acid accumulates in blood preservatives because of a closed system. Little ability to metabolize and exchange carbon dioxide occurs, although some transport of gases does occur through plastic bags. In whole blood, initial values of 13.8 ± 2.1 μ moles/gHb serum levels of lactic acid were measured. By day 2 of storage, a statistically significant increase in lactic acid had occurred. This increased during the entire storage interval studied to approximately 10 times the baseline value by day 18 of storage. In packed red blood cells, a lactic acid increase of statistical significance was found by day 2 following donation. The level increased approximately seven times by day 18 of storage.

Whole blood appears to produce lactate at a significantly higher rate ($p<0.001$) than packed cells. The presence of a glucose substrate and plasma may allow an increased rate of metabolism in whole blood.

The lower evolution of lactic acid in packed red blood cells may be due to the compression occurring during the packing procedure in a relatively inert environment. Approximately 10 times as much lactate acid was produced by day 18 of storage of whole blood as compared with a sevenfold increase in packed red blood cells. Absolute values as well as percentages increase much more rapidly in whole blood than in packed cells. This may represent the presence of more glucose substrate in whole blood than in packed cells. In addition, the packing process may result in the cells being more inert with lowered metabolism.

pH

All blood preservatives maintain and increase acid pH during preservation. A putative advantage of citrate phosphate dextrose over acid citrate dextrose is the somewhat higher pH values. The higher pH has been one of the factors held responsible for the better maintenance of diphosphoglycerate. In the whole blood bags analyzed, the mean pH was 7.0 $\pm$ 0.08 on day 1. By day 18 of storage this had dropped to 6.68 $\pm$ 0.2 pH units. A statistically significant drop in pH had occurred by day 7 and continued the usual increase in acidity during the period observed. The pH of all bags of citrate phosphate dextrose mixed with blood was somewhat lower than other observers have reported.

The packed red blood cells had a pH of 6.9 $\pm$ 0.05 on the day of collection after elution from the tripack. By day 4 of storage a statistically significant drop in pH to 6.81 $\pm$ 0.05 had occurred. Throughout the entire period of comparison (see graph), pH fell faster in packed cells than in whole blood. A statistically significant difference in the role of change of pH was found throughout the entire period observed.

Saturation

In 1958, Valtis and Kennedy described an increasing saturation of blood in acid citrate dextrose preservative per unit time. This has subsequently been attributed to 2,3 DPG loss during the storage interval. An increase in saturation per unit of time was found in both packed red blood cells and in whole blood. These changes correlated significantly with the 2,3 DPG values. (No difference between packed cells or whole blood was found.)

Calcium

Calcium is a cation which maintains the integrity of the cell. The initial calcium level of 7.8 $\pm$ 0.35 mg/100 ml in the four units of citrate phosphate dextrose preservative was longitudinally studied.

A calcium value of 8.23 ± 0.17 mg/100 ml was found at the end of the storage interval. This did not represent a statistically significant difference from the initial value.

In packed cells the initial calcium value was 8.18 ± 0.25 mg/100 ml. This had significantly increased by day 11 and persisted at an elevated level to the end of the storage interval. In addition, after day 4, calcium was significantly higher ($p<0.001$) than in whole blood throughout the entire storage interval. The elevated calcium levels could represent hemolysis of cells during the sampling intervals.

Inorganic phosphate

Inorganic phosphate is known to increase in ionic concentration during storage intervals of most blood preservatives. In whole blood preserved in citrate phosphate dextrose, increased amounts of inorganic phosphate is present when compared with acid citrate dextrose. An initial inorganic phosphate value of 10.16 ± 0.83 mg/100 ml increased to 14 by the end of the storage interval. It first rose significantly above normal on day 7. In packed red blood cells the initial inorganic phosphate value was 10.25 ± 0.4 mg/100 ml. By day 9, it was above normal.

Inorganic phosphate levels increased significantly more rapidly in packed cells than in citrate phosphate dextrose preservative ($p<0.001$), and the overall level was higher in packed cells ($p<0.001$).

Microaggregates

Evidence is abundant from many investigators participating in this conference that particulate matter consisting of red cell ghosts, fibrin and platelet particles, and perhaps plasticizers, increase in abundance with duration of blood storage. In the broadest sense, microaggregate formation is an intimate part of the storage lesion of liquid preserved blood.

In vivo studies of transfusion

The patient-transfusion interface and subsequent homeostatic adaptation is complex and important. A retrospective study was performed on 44 trauma victims who had received 90% or more of their estimated blood volume within a 24-hr period of time. The data are from 48 patients who received approximately one-half of their transfusion with blood products preserved in acid citrate dextrose preservative and the other half in citrate phosphate dextrose preservative. The metabolic data, which include P_{50}, DPG values and lactic acid levels, are part of patient monitoring in massive transfusion in our institution.

The P_{50} of the oxy-hemoglobin dissociation curve was measured by equilibration of whole blood in tonometers with three gas mixtures containing 5% carbon dioxide, nitrogen, and three oxygen tensions which approximated 17, 22 and 29 torr. The blood was subjected to a 20-minute tonometer-equilibration period and then standardized for oxygen tension saturation and pH. Results were normalized at standard conditions of 38°C, a P_{CO_2} of 40 and a pH 7.4. The Hill equation was then fitted to these three points near the half saturation point to derive the P_{50} of the oxy-hemoglobin dissociation curve and the results were expressed in torr.

Diphosphoglycerate (2,3 DPG) was measured using a Sigma kit and expressed in micromoles per gram of hemoglobin. Whole blood lactate was measured with the Boehringer kit and expressed in micromoles per gram of hemoglobin. The results of the *in vivo* studies (Fig. 3) demonstrate a remarkable homeostatic adaptation for the clearance of lactic acid and the normalization of the P50 of the oxy-hemoglobin dissociation curve (Table 3). Regardless of the preservative used, the mean P_{50} immediately following transfusion was 24.3 $\pm$ 3 mmHg with 2.3 DPG values of 14.9 $\pm$ 6.2 μ moles/gHb.

There was no statistical difference in 24 hr from the initial measurement in DPG and P_{50} values. At 48 hr, red cell function was normal or above normal and there were no signigicant differences between the groups.

Correlation between P_{50} and 2,3 DPG values was statistically significant (r = 0.91, p less than 0.01). Patients receiving ACD blood had higher lactate values than CPD recipients immediately following transfusion. (48.4 $\pm$ 36.5 vs 28.1 $\pm$ 16.3 μM/gHb.) At the end of 24 hr posttransfusion, normal lactate acid levels were present in both groups.

Perhaps the most significant finding in the study was the mean age of storage life, which was 4.6 for $\pm$ 3.9 days. Obviously blood of this short shelf life, regardless of preservation in CPD or ACD, has little deterioration in 2,3 DPG values. Blood less than 5 days of age would be expected to have minimal microaggregates, also.

DISCUSSION

The transfusion of stored blood with its many preservative alterations results in metabolic alterations which conceivably increase endogenous microaggregate formation and cause thromboembolism. Cellular hypoxia may result from transfusion-related metabolic abnormalities which increase the affinity of hemoglobin for oxygen and potentially impair tissue oxygen tension release.

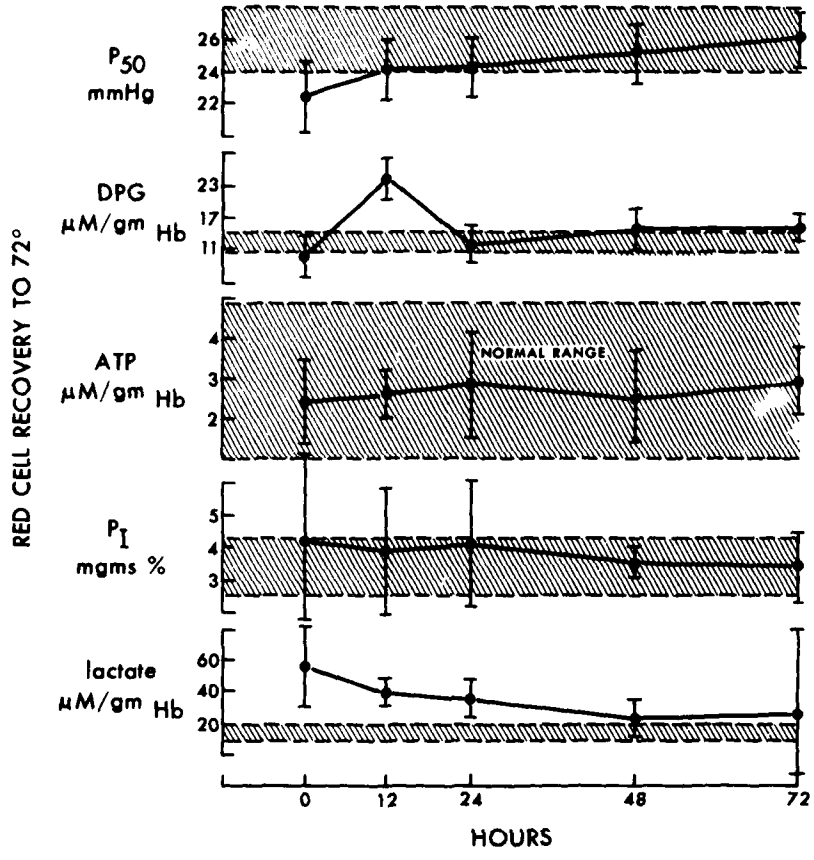

Fig. 3. Data from patients with massive transfusion. Rapid recovery of red cell 2,3 DPG occurred with clearing of lactic acidosis within 24 hr. By permission, Journal of Trauma, 1975.

Respiratory alkalosis occurs secondary to hyperventillation, following hemorrhage. Transfused patients are cold from ambient temperature loss and from the transfusion of blood preservatives at 4°C. The metabolism of citrate moiety of liquid-preserved blood produces a metabolic alkalosis. The combination of respiratory and metabolic alkalosis, hypothermia, and increased hemoglobin affinity for oxygen secondary to low levels of 2,3 DPG are factors which are additive in producing a high affinity of hemoglobin for oxygen which could impair release of oxygen from the red cell. Data for our laboratories suggest that tissue oxygen tension is reduced after massive transfusion and does not return to normal until 3-4 days after the 2,3 DPG values and pH changes have returned to normal.

TABLE 3. METABOLIC DATA FROM 44 TRAUMA PATIENTS WHO HAD 90% OR MORE OF THEIR BLOOD VOLUME REPLACED BY PRESERVED BLOOD BECAUSE OF INJURY. THE DATA INDICATE NORMALIZATION OF 2,3 DPG, P_{50} AND LACTATE VALUES BY 24 HR. IN THE STUDY, THE MEAN AGE OF BLOOD ADMINISTERED WAS 5 DAYS.

	Immediate		24 hr		48 hr	
No. of patients	ACD 24	CPD 20	ACD 24	CPD 20	ACD 24	CPD 20
P_{50} (26.1±1)	24.28	24.25	24.50	23.98	26.17	24.55
mmHg	±2.29	±1.36	±1.93	±1.88	±2.07	±1.49
2,3 DPG (14.2± 1.3)	14.80	13.98	13.40	13.28	15.63	14.29
μ moles/gHb	±6.40	±2.27	±4.46	±2.01	±4.23	±2.39
lactate (15.0± 2.0)	48.4	28.1	24.1	20.1	35.0	17.0
μ moles/gHb	±36.5	±16.3	±11.4	±10.5	±37.5	±8.8
% blood volume	96.9	93.4	16.1	15.1	9.4	11.3
transfused	±8.1	±9.0	±18.1	±17.3	±15.0	±19.5
age of blood	5.2	4.1	2.6	5.9	2.3	3.0
(bank days)	±4.1	±3.8	±1.8	±3.1	±0.8	±1.5

By permission, Journal of Trauma, 1977.

Prolonged shock, coupled with altered oxygen transport, may contribute to endogenous generation of microaggregates. Microaggregates from preserved blood produce a source of microemboli, also. Both may cause some degree of lung damage.

Determining the relative importance of microaggregates, endogenously produced or from transfusion, in adult respiratory distress syndrome, is difficult. Pulmonary failure after injury is a complex syndrome with many causes.

Current blood filtration practices during most transfusions are probably adequate. No proved physiological alteration in pulmonary or other organ function occurs in low volume blood transfusion.

The greatest enthusiasm for finer filtration of transfused blood is in massive transfusion. The initial Dacron-wool filters, however, remove platelets as well as microaggregates, which limit the enthusiasm for filtration. Dilutional thrombocytopenia frequently occurs in massive transfusion and one object of early hemotherapy is to provide whole blood with functioning platelets, as well as platelet concentrates. Improvement in filter design has minimized the loss of platelets by filtration while continuing to remove microaggregates.

The logistics of massive transfusion are important when the value of filtering blood products are considered. The recipient of multiple units of stored blood is transfused initially by blood bank policy with blood approaching the end of its shelf life, which predictably has high concentration of microaggregates. Filtration, if useful, would best be used for the initial, older units of blood. Because massive transfusion depletes blood bank resources, the mean age of blood received in massive transfusion is usually low (5 days in our series). Blood which has been stored less than 8 days probably does not require fine filtration. Whether administration of blood without fine filtration produces pulmonary damage which evolves into adult respiratory distress syndrome is undetermined.

Microaggregates which occur *in vivo* during shock may be a central mechanism through which pulmonary failure occurs following injury and transfusion. The microaggregates which have been documented to occur in preserved blood during the storage interval may be removed by fine filtration. Microaggregates, 2,3 DPG, lactic acid, *etc.*, which increase during liquid preservation of blood are of minimal clinical significance.

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THE ROLE OF MICROAGGREGATES IN THE RESPIRATORY DISTRESS SYNDROME

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INTRODUCTION

Multiple factors have been implicated in the etiology of the respiratory distress syndrome, but none has been shown to be uniquely responsible. Oxygen toxicity, barotrauma, neurogenic factors, fluid overload, and vasoactive humoral factors all have their advocates. While each of them may have a role in selected patients, it has not been our experience that any of them can be consistently identified with the development of respiratory distress, and we have sought an alternative explanation.

Our first experience with the respiratory distress syndrome (1) occurred approximately 10 years ago in a group of patients who were undergoing major vascular surgery with bypass grafting of the distal aorta, iliac, and femoral arterial systems. Postoperative respiratory distress developed in a significant number of these patients, even when no preexisting lung disease was present and no specific inciting cause could be identified. A common feature of this population was the need for aortic cross clamping for a period of time during the operative procedure with hypoperfusion of the lower extremities. This observation led to the concept that the period of low perfusion of the legs resulted in the development of thrombi due to stasis in the capillary circulation. With reperfusion after bypass grafting the small capillary thrombi would be washed out and carried to the lung, where the pulmonary capillaries would act as filters resulting in diffuse embolic occlusion of small pulmonary vessels.

In an attempt to study this phenomenon an animal model was developed by Lim et al. (2) to duplicate the essential features of the human situation. In a series of canine experiments, the infrarenal aorta was cross clamped and collateral arterial circulation to the hind limbs was ligated. This resulted in a perfusion pressure of less than 10 mmHg in both femoral arteries. The cross clamping was maintained for 4 hr and was then released with reperfusion of the hind limbs. When this experiment was carried out in 24 dogs, all developed severe respiratory insufficiency with findings identical to the human situation and the mortality rate without treatment was 50%. The earliest pathological finding was the presence of embolic fibrin and platelet material in the pulmonary arterioles. Sequential changes which occurred during the first 24 hr consisted of

increasing periarterial hemorrhage and edema, increasing interstitial hemorrhage and edema and progressive consolidation.

Two further canine experiments (3) were done to confirm that the embolic material arose in the lower extremities and was carried to the lungs rather than being a humoral factor in the circulation. In the first experiment, the superior vena cava was ligated just above the right atrium and the right pulmonary artery was anastomosed to the superior vena cava (Glenn procedure). This resulted in blood flow from the superior vena cava being directed into the right lung while flow from the inferior vena cava passed through the right heart in normal fashion and entered only the left lung. Aortic cross clamping and release after 4 hr was carried out as previously described, but the pathologic changes occurred principally in the left lung and the right lung appeared to be protected. Sections taken after 24 hr demonstrated minimal congestive changes in the right lung while the left lung showed the marked hemorrhagic and congestive changes previously described.

The second experiment to verify the embolic nature of the pulmonary dysfunction was transposition of the inferior vena cava and portal veins, so that blood returning from the distal inferior vena cava flowed into the portal vein and liver before reaching the right heart while portal blood flowed directly into the proximal inferior vena cava and the right heart without passing through the liver. When the aorta was cross clamped under these circumstances, and released after 4 hr, both lungs were spared and the animal did not develop the pathologic changes described. It was concluded from these experiments that when respiratory failure develops after procedures requiring aortic occlusion a direct embolic cause could be substantiated.

Respiratory failure after trauma.

In the late 1960's and 1970's the increasing incidence of civilian violence led to increased numbers of severely traumatized patients brought to San Francisco General Hospital, and the occurrence of respiratory distress in trauma victims became more evident. Respiratory failure in this population rarely developed unless the patient had either severe soft tissue trauma, extensive trauma with long bone fractures, severe head trauma, or massive blood loss and prolonged shock. In this group of patients a study was done (4) to see of pathologic sections disclosed evidence of embolic material as was seen in the vascular surgery population. The problem of proving or disproving thromboembolic causes of pulmonary dysfunction are hampered by the difficulty of showing the presence of emboli within the circulation and the difficulty of obtaining pulmonary tissue for study other than by autopsy. There are no specific

function tests which define the presence of microembolism, and the best theoretical test, the dead space to tidal volume ratio, is virtually always elevated in acute respiratory failure.

With the assistance of the San Francisco Coroner's Office, a large number of lung sections were examined from patients dying at various times after major trauma. These sections disclosed the same changes which had been seen previously both in animal experiments and the vascular surgery patient population. Embolic occlusion of small pulmonary vessels was common in patients dying within the first 24-48 hr of their injuries whether as a result of massive trauma or prolonged shock due to hemorrhage. In patients who survived for longer periods, progressive vascular congestion followed by interstitial and intraalveolar hemorrhage and edema were seen. When patients survived for a few days they developed hyaline membranes within the alveoli and very commonly would develop the picture of bronchopneumonia with inflammatcry cells in the alveolar spaces and interstitium. In patients who survived for 10 days or more, the presence of pulmonary fibrosis was increasingly evident (Fig. 1).

Microscopic pathology.

Microemboli	0-24 hr
Vascular congestion	12-36 hr
Interstitial edema and hemorrhage	24-48 hr
Intraalveolar edema and hemorrhage	24-72 hr
Hyaline membranes	5 days
Bronchopneumonia	10 days
Pulmonary fibrosis	28 days

Fig 1. A summary of postmortem microscopic changes in lung sections at various times after traumatic injury.

In most cases the embolic material stained positively for fibrin and platelets although in selected situations embolism of other tissue was found. In a young woman 8-months pregnant who sustained severe blunt trauma to the chest and abdomen, amniotic fluid embolus to the pulmonary arterioles was demonstrable in a lung section removed immediately after the injury. At the same time the patient had fulminant acute respiratory failure. In another instance a patient with severe closed head injury and acute respiratory failure was shown at postmortem to have embolization of cerebral tissue to the pulmonary microcirculation.

The classical type of tissue embolism, which has been recognized for several decades, is fat embolism after long bone fractures. Studies have never clearly resolved the issue over origin of the fat and some believe it is tissue embolism from marrow cavities, while others believe it results from the release of fat from lipoproteins in the blood. In one of our patients who died 5 days following bilateral femur fractures, fragments of bone marrow could be seen occluding pulmonary arterioles at autopsy. In two other patients who had stable pulmonary function several days after injury, sudden deterioration in arterial oxygenation was noted immediately following orthopedic manipulation of their fractures. These examples tend to confirm that at least a portion of the embolism can arise from marrow cavities.

Although the above forms of tissue embolism account for a certain number of patients with respiratory failure, most have no specific source demonstrable yet still show evidence of fibrin and platelet thrombi in pulmonary arterioles at autopsy. In this group of patients it is our feeling that intravascular aggregation of platelets and intravascular coagulation are responsible for the production of emboli on the venous side of the circulation which are filtered in the lungs.

OTHER STUDIES

Platelet aggregation.

Several investigators have demonstrated the presence of platelet aggregates following trauma and shock. Robb et al. (5, 6) found in rabbits studies with cinephotography that if hemorrhagic or endotoxic shock were induced, emboli could be seen arising from the microvasculature of the bowel wall. After trauma the emboli could be seen arising from the area of the injured tissue. Studies of the pulmonary circulation showed that the emboli were filtered in the pulmonary circulation and platelet counts dropped while the platelets were being sequestered in the lungs. Berman (7) studied venous drainage of the thigh following gunshot wounds, and found platelet aggregates coming from the injured extremity. Saggau et al. (8) introduced standardized bone trauma to the hind limbs of dogs and studied three groups. Group 1 was subjected to trauma alone, group 2 to hemorrhage alone and group 3 to trauma plus hemorrhage. All animals in group 3 died and those in groups 1 and 2 survived. Examination of the lungs in the latter groups showed disseminated pulmonary thrombosis. Bo and Hognestad (9) injected collagen extract into a cat and found that this produced an increase in pulmonary vascular resistance which was reversible. When lung sections were taken at the peak of bronchoconstriction, platelet aggregates were found in the sections.

Other investigators have studied the effect of platelet aggregation on lung function and documented the harmful effects when platelet aggregation occurs. Thomas et al. (10) embolized autologous clots to the lungs in rabbits and noted the accumulation of platelets on the embolus and the broncho-constriction which resulted. He found that heparin given prior to embolization prevented the aggregation of platelets to the clot and resulted in negligible bronchoconstriction. Radigan (11) infused thrombin in dogs and simultaneously injected radioactively labelled platelets. These were also found in the lung and were associated with the development of pulmonary vasoconstriction. He found that prior administration of aspirin prevented the vasoconstriction and concluded that some type of humoral substance was released by the platelets and was responsible for the vascular spasm. Magilligan et al. (12) found that if methylsergide, an antiserotonin agent, was given prior to embolization, the compliance changes following embolization would decrease. Stein and Thomas (13) produced airway constriction in dogs by injecting endotoxin. They found that the prior administration of heparin, the administration of a serotonin antagonist or the induction of thrombocytopenia protected against airway constriction. If the platelet count was above 50,000, endotoxemia resulted in bronchoconstriction; below this level it did not.

It appears from these studies that platelet aggregation and embolization can result from hemorrhagic shock or traumatized soft tissue. These aggregates travel through the venous circulation to the lung where they are filtered by the pulmonary capillary bed. Within the lung, the platelet aggregates appear to release humoral factors which produce bronchoconstriction and vasoconstriction and may produce increases in capillary permeability.

Intravascular coagulation.

More generalized coagulation changes after trauma have also been noted by a number of investigators. Innes and Sevitt (14) first called attention to the acceleration of coagulation in injured patients with accompanying fibrinolysis. They documented the multiple clotting changes which occurred in 42 patients following the traumatic event, and noted the occurrence of pulmonary thrombi in patients dying within the first 2 days after injury. Eeles and Sevitt (15), in a pathological study of 185 injured and 135 burned patients, found that capillary thrombi in the lung were common, being present in 25% of the injured and 29% of the burned patients. They were found most often when the patient died within 3 hr of the injury, and their incidence increased in proportion to the severity of the injury.

Simmons et al. (16) reported on casualties in Vietnam and found that the severely wounded had hematologic changes compatible with disseminated intravascular coagulation. Half of the patients he studied showed changes in the prothrombin time or partial thromboplastin time after injury, and the more seriously injured patient had the greater changes. Busch et al. (17) studied six traumatized patients with radioactively labelled fibrinogen and found that two, who developed respiratory distress, had fibrin demonstrable in the lungs by radioactive tag. Milligan et al. (18) reported on 20 patients with septic shock, and found that 19 had evidence of disseminated intravascular clotting, and all but 1 had a significant decrease in arterial oxygen tension. Most patients also had severe radiologic changes and these findings suggested to him that there is an association between the hematologic and pulmonary complications.

In addition to the clinical studies, there have been animal studies which support the connection between the intravascular coagulation and the pulmonary lesion. Arfors et al. (19) infused thrombin and a fibrinolysis inhibitor, aminocaproic acid, into dogs and produced all the changes of "shock lung" including pulmonary failure. Thrombin injected alone caused minimal changes and previous defibrination of the animal prevented respiratory failure. Thrombocytopenia did not prevent the pulmonary insufficiency and the authors concluded that the results showed that the presence of platelets or leukocytes is not necessary for the development of pulmonary damage, and that pulmonary retention of fibrin alone can induce it. Fibrinolysis, if unimpaired, provides adequate protection in the experimental circumstances, and radioactively labelled fibrinogen which appears in the lung is rapidly removed without producing lung damage in the presence of normal fibrinolysis (20).

Olsson et al. (21) found that the injection of thrombin in dogs resulted in significant respiratory effects as evidenced by a fall in compliance and a fall in arterial oxygen tension. Aspirin prevented the respiratory effects and it was postulated that prostaglandin production was the mediator of lung damage. He also found in subsequent studies, that during the production of posttraumatic respiratory insufficiency, platelets were consumed as the pulmonary vascular resistance increased. He, therefore, felt that the platelet-release reaction was involved in smooth muscle stimulation which affected respiratory function.

Lindquist (22) noted that lung lymph flow increased markedly after thrombin infusion into dogs, and found that an increase in pulmonary vascular permeability had occurred. On microscopic examination, interstitial edema was also noted in the kidney and liver.

In our own series of patients in the Intensive Care Unit a correlation was made between the severity of the respiratory failure which occurred and the presence of abnormalities in the clotting studies. These results are shown in Figure 2. It is evident that the correlation between the severity of respiratory failure and the severity of intravascular coagulation occur in parallel, and tend to support the thesis noted above, that coagulation plays a role in the respiratory distress syndrome (23).

	Coagulation changes		
	Severe	Moderate	None
Severe	12	1	0
Moderate	1	13	2
Negative	0	1	10

Fig 2. Correlation of clinical morbidity and coagulation changes in 40 patients after traumatic injury. Clinical morbidity was considered severe if more than 3 days of mechanical ventilation was required, moderate if 6 hr-3 days of mechanical ventilation was required, and negative if less than 6 hr of ventilation was needed. Coagulation changes were severe if more than five parameters were abnormal, moderate if two to five were abnormal, and negative if one or none were abnormal.

CURRENT RESEARCH AND FUTURE PROJECTIONS

Although extensive evidence exists in both clinical and experimental studies for the connection between intravascular coagulation and the respiratory distress syndrome, a conclusive relationship has been difficult to prove. This is the result of two factors: First, the occurrence of the pulmonary lesion itself can be inferred from physiologic studies, but there are no specific tests which allow one to define the presence of microembolism. Most pulmonary insults, such as aspiration pneumonia, pulmonary contusion, and cardiogenic pulmonary edema result in similar functional physiologic changes in the lung. The pathologic studies described previously allow one to categorize the nature of the lung lesion after death, but there are no suitable histologic means of following its development on a sequential basis, or evaluating sublethal levels of pulmonary embolism.

The second difficulty is related to the problem of defining intravascular coagulation. The classical tests which are used in diagnosing this condition are a fall in the platelet

count, a rise in prothrombin time and partial thromboplastin time, the presence of soluble fibrin monomer in serum and the presence of fibrin degradation products. Unequivocal evidence of intravascular coagulation, as evidenced by abnormalities in all the studies, is usually present only in extensive and fulminant intravascular clotting, which can also be diagnosed on a clinical basis by the presence of diffuse oozing from venipuncture sites and incisions. Lesser degress of intravascular coagulation, which are manifested only by equivocal changes in the clotting studies mentioned, are difficult to diagnose. The situation, therefore, exists wherein the two clinical entities which are being correlated - pulmonary microembolism and intravascular coagulation - are both difficult to diagnose on objective grounds.

A solution to one portion of this problem has been the development of a means of assessing fibrinogen catabolism. The use of radioactively labelled I-125 fibrinogen has been carried out in Europe for approximately 10 years, but its introduction in the United States is recent. Prior to the availability of commercially tagged fibriongen, it was necessary to isolate autologous fibrinogen from the patient, label this, and then reinject it into the patient. With the advent of commercially tagged fibrinogen of good quality, the process has been considerably simplified. The isotope can be injected directly and the decay rate followed by daily assessments of residual activity in the serum. Under normal circumstances the fibrinogen decay occurs in logarithmic fashion and can be described by a half-life which in normals is approximately 100 hr. When intravascular coagulation occurs, fibrinogen is consumed and a more rapid decay of radioactivity occurs which can be described by a shorter half-life. Severe degrees of intravascular coagulation are manifested by half-lives of less than 30 hr, while an intermediate level of intravascular coagulation is seen with half-lives of between 30 and 50 hr.

Figure 3 shows the pattern of fibrinogen decay in a normal patient. After an initial period of equilibration, a constant logarithmic decay rate was established with a half-life of 104 hr. Figure 4 shows a decay curve for a 28-year-old female, 2 weeks after multiple blunt traumatic injuries. At this point she had become systemically septic due to an intraabdominal source, and had equivocal findings for intravascular clotting. Her fibrinogen half-life on successive days was 13, 34 and 23 hr. The initial day's measurement is unreliable because of third space equilibration which takes place in the first 24 hr, but measurements on days 2, 3 and 4 after injection all demonstrate a consistent shortening and indicate that fibrinogen consumption is increased three- to fourfold in this patient. In spite of this, serum fibrinogen levels were normal, platelet count was

greater than 50,000, fibrin monomer was only 1+, and prothrombin and partial thromboplastin times were elevated only by 20% over control values. This has been a common finding in septic patients, and leads us to believe that sepsis is one of the more potent activators of the clotting system while conventional hematologic tests inadequately indicate the occurrence of intravascular clotting.

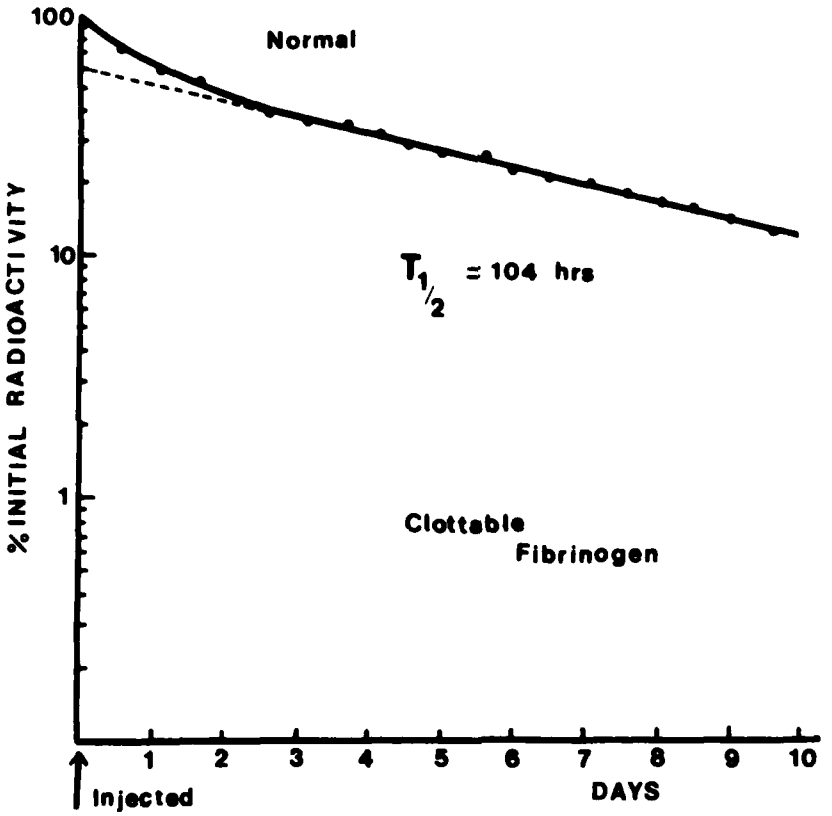

Fig 3. I-125 fibrinogen decay curve in normal volunteer showing half-life of 104 hr.

In a limited number of patients, the presence of intravascular coagulation with fibrinogen half-life shorter than 30 hr has shown a high correlation with respiratory failure and failure of other organs. In a series of 19 patients, 6 who had fibrinogen half-lives less than 30 hr had a 67% mortality. Five who had fibrinogen half-lives between 30 and 40 hr had a 40% mortality, and eight who had fibrinogen half-lives of greater than 50 hr had no mortality. These studies are only in their early phases and no definite conclusions have yet been reached, but it does appear that the use of this new modality will enable

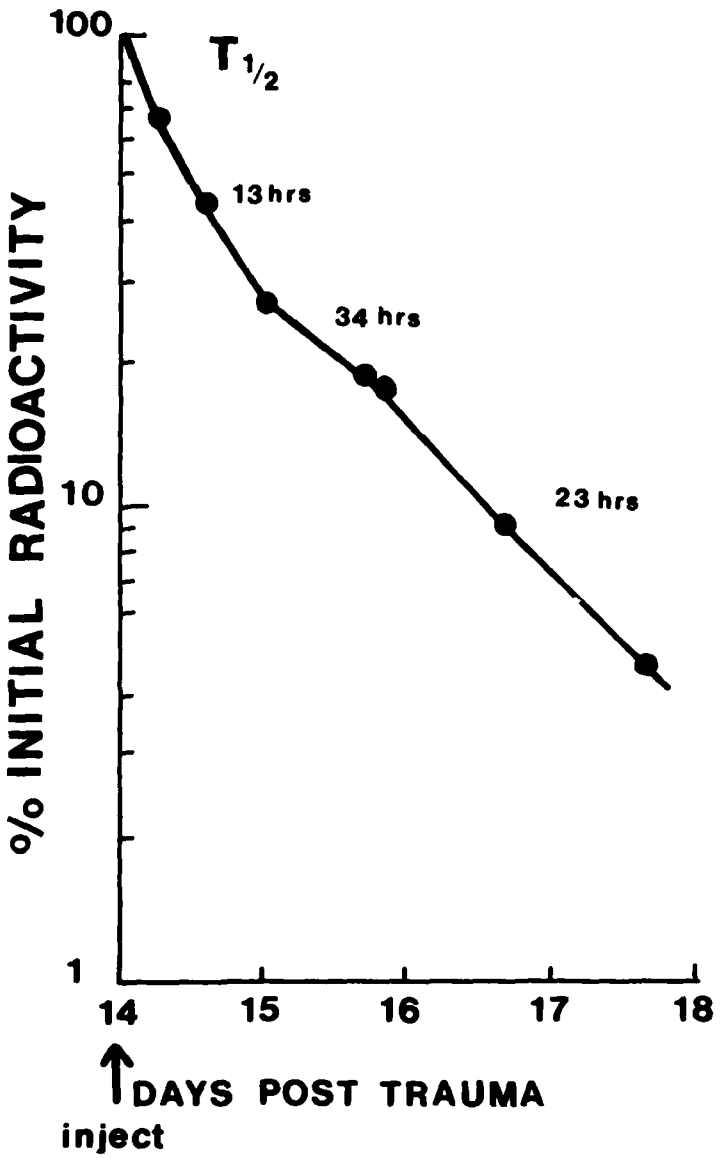

Fig. 4. I-125 fibrinogen decay in a patient with sepsis 2 weeks after traumatic injury. Shortened half-life during first 24 hours after injection is partially due to third space equilibration. Remaining three days of study show half-lives of 1/4 to 1/3 normal, documenting increased fibrinogen catabolism.

the degree of intravascular coagulation to be more precisely quantitated than has heretofore been possible. It also provides a means for monitoring heparin therapy, as prolongation of a shortened half-life is evidence of adequate heparin effect with restoration of fibrinogen catabolism to normal.

It is hoped that with the combined use of this new assay of clotting, plus the availability of more sophisticated tests of pulmonary dysfunction, a more direct cause-and-effect relationship may be established between the presence of intravascular coagulation and the development of respiratory dysfunction.

SUMMARY

Evidence from animal experimentation which indicated a connection between pulmonary thromboembolism and acute respiratory failure has been described. In traumatized patients, the pathologic evidence for an embolic nature to the respiratory distress syndrome has also been documented. Clinical and experimental evidence that intravascular coagulation is associated with respiratory failure has been presented, and the problems with previous attempts to establish a connection between these two described. The current methods for assessing intravascular coagulation using radioactively labelled fibrinogen to establish a half-life in the serum have been discussed, and a potential use for this test in objective quantitation of intravascular coagulation has been described. It is hoped that use of this new assay, plus more sophisticated tests of pulmonary function may allow a direct causal relationship to be established in the future for the role of intravascular coagulation in respiratory failure.

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SUMMARY

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The one point upon which all participants appear to agree is that microaggregates are present within the blood bag, and if they are transfused without a fine pore filter, they are infused into the patient. In addition, no studies have been presented which claim that microaggregates are beneficial.

If microaggregates are present, if they are not beneficial, and if it were cost free to remove them, we would do so. The question then becomes one of cost, both in money and in time. In St. Louis, ultrafiltration adds an additional 2 - 3 dollars to the cost of 1 unit of whole blood. In 1975 there were 7.5 million units of blood transfused in the United States. If we contemplate ultrafiltration of all transfusions, there is a significant amount of money involved. But more significant is the expense in time. The massively transfused patient is the one in whom we would recommend the use of fine filters, which then results in two choices. We can select a filter which is inefficient and which can tolerate multiple passages of blood through it with minimal filtration. We then allow some of the particles through so that we can deliver the blood to the patient more rapidly, because we all recognize that the patient's survival may depend on rapid blood volume replacement. It is unlikely, however, that such a choice really prevents micro-embolization. The second choice is to use a very efficient filter which will unquestionably slow the rate of transfusion.

In my opinion, the dilemma in cost is the time element. Any expense is worth it if we get a proportional patient benefit. The benefit that is being proposed is the prevention of micro-emboli. In order to define what we are preventing, we need to know what to measure, when to measure it, the correct population of patients in which to measure it, and we must relate it both to the volume and the age of blood that is being transfused.

The ideal study has not been done, and it probably never can be done. Further studies in man are certainly needed. The desirability of further studies in animals is questionable. It is quite clear that the baboon studies all yield negative results. Dogs seem to yield mostly positive results, but even this is not completely agreed upon. The species differences are so great that we are probably wasting money by continuing further animal studies.

The question of what to measure has not yet been answered. Initially, it was assumed that measurement of arterial pO_2 was the answer, but there is fairly good evidence that any form of microembolization does produce immediate hypoxemia. Whether the injected material is air, tiny seeds, firm glass beads, fat, or any form of embolic material that forms microemboli to the pulmonary capillary bed, it has been found to produce hypoxemia and to produce it almost immediately after injection. Additionally, the degree of hypoxemia is proportional to the amount of material that has been injected. There are almost certainly changes in pulmonary vascular resistance with microembolization and again these changes occur immediately. Whether or not there are changes in pulmonary compliance is unanswered. Compliance is not a very noninvasive method of monitoring the patient, but then neither are pulmonary vascular resistance measurements.

Finally, we need studies on the histology of human lungs after massive transfusion that are better controlled. We need studies on patients with similar injuries who receive differing amounts of blood in order to determine whether the particles we are seeing are coming from the blood or from the crushed extremity, intraabdominal injury, or other source.

Since there remains question about what to measure, do we at least know when to measure it? I have indicated above that some of the changes occur very rapidly. But all of these immediate changes relate to the mechanical effects of embolization. There is a real and significant question as to whether there is a second phase of effect from the embolization of this transfused material. There may be something that produces damage to the pulmonary capillaries and may then be manifested after an interval of 1 - 3 days, perhaps causing a secondary functional deterioration. It would, therefore, seem that we should study patients for at least several days following transfusion. Certainly, emphasis should be placed on the early period and then at varying time intervals over the next several days.

What population of patients should we study? Ideally, we should study normal patients, with no underlying disease or other misleading factors, but obviously these are not the types of patients who require massive transfusion. Every injury that necessitates massive transfusion is also prone to produce other pulmonary effects unless we are very careful and moderately lucky in caring for such patients. I concur with the opinions of others who have spoken here - this disease seems to be disappearing. I do not feel that anyone can identify what

particular aspect of patient care is responsible for the disappearance of the problem. I do not believe that anyone can say that it is filtration, since the decrease is occurring in patient populations which receive unfiltered as well as filtered blood. At least filtration is not the sole answer.

Finally, the volume of blood that is transfused is obviously important. There are porentially enough microemboli in 4 units of blood to occlude two-thirds of the capillaries feeding alverlar units in the lungs, and that much occlusion should produce detectable changes. Something on the order of 4 - 5 units of blood should be the critical point at which significant microembolic problems arise. The age and type of blood transfused is also important. Blood which has had the buffy coat removed may act entirely differently from blood which has it included.

Are there alternative methods for solving the problem? We are entering an era of better management of blood as a resource. Apart from the microemboli, there are other materials in the plasma fraction which are bad for the patient. In fact, on most of these factors, there is no disagreement. There is antigenic debris, mechanical debris, metabolic debris, and infectious agents. The patient would benefit from not receiving any of these things, since what they really need is the red cells. What is needed is a method for removing all of the plasma and non-red cell material and just giving patients the necessary component for their particular needs.

Already on the horizon is the use of adenine-preserved blood, which may make the problem of microaggregation even greater. However, we are also entering the era of conserving platelets and not including them in the standard transfusion, but instead saving them for specific indications. Once the problem of preserving platelets for more than 48 hr is solved we may well see routine removal of platelets from all blood collected in the United States. Once that is done, we will not only have minimized the microaggregate problem, but we will have improved the whole system. Washing red cells would be an even better solution, because that gets rid of almost all of the plasma and may even get rid of most of the transmitted infectious diseases. I am not certain that the cost-benefit analysis which has been used as an argument against washing cells is legitimate because the clinical gain is potentially quite large.

What can we conclude at this point? I think there has been a good case made for using the fine filters in cardiopulmonary bypass for two reasons. First, I think that it is a different situation from the massively transfused patient, since changes

occur both in the blood of the patient and in the transfused blood. Secondly, the material is infused directly into the systemic circulation without the benefit of pulmonary capillary filtration, so that the effects of microaggregates are on the brain and other vital organs, and not on the lung. I think it is also advisable to use the ultrafilters in the case of people who are extensively transfused. It concerns me that the debris is present and that it is not doing any good and it is being infused into the patient. If we can remove it at the cost of a few extra dollars per unit of blood, while limiting the population only to those who we think will get a large volume of blood (greater than 5 units), then I think it is well worth it. However, if doing this is going to delay transfusion in a patient who is actively bleeding, I have no doubt at all that it is far better to get the blood without the delay.

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